CO-CULTURE SYSTEM CONSISTING OF GENETICALLY ENGINEERED *Synechococcus elongatus* AND *Escherichia coli* W

by

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A thesis submitted to The Johns Hopkins University in conformity with the requirements for the degree of Master of Science in Engineering

Baltimore, Maryland

June 2019

ABSTRACT

Co-culture has been a rising topic in microorganism research field and it has great potential in industrial production. Thus, an attempt is made to build a co-culture system with two model organisms, *Synechococcus elongatus* cscB, which is a genetically engineered sucrose producing strain, and *Escherichia coli* ATCC 9637 and both have great potential in industrial application.

After medium modification, a co-culture system is built, and it reveals that there are additional stresses on both organisms in co-culture systems. High amount of phosphate is added into the modified medium to meet *Escherichia coli coli* ATCC 9637 growth requirement. In such environment, pH plays a major role in sucrose production of *Synechococcus elongatus* cscB; phosphate addition shows toxic effects on *Synechococcus elongatus* cscB but improve its sucrose production; and co-culture shows both positive and negative effects on both organisms and suggests potential metabolic changes in both organisms.

The conclusion is that a co-culture system consisting of *Synechococcus elongatus* cscB and *Escherichia coli coli* ATCC 9637 is feasible but may not be favorable.

Primary Reader and Advisor: Dr. Michael Betenbaugh

Secondary Reader: Dr. Marc Donohue

ACKNOWLEDGEMENTS

I would like to thank Dr. Mike Betenbaugh first for taking me as a master student, giving me such a great opportunities to do researches, and most importantly his advisory in my research project. His devotion and perfection in scientific research and great personality will be my model in future. I deeply appreciate his guidance and encouragement during my study at Johns Hopkins University.

Also, I would like to thank Tinting Li, Liqun Jiang, Chien-ting Li, and Qiong Wang from Betenbaugh lab, Steve Chow and Christopher Brueck from Bouwer Lab of Environmental Health and Engineering department, Eric Sakowski from Preheim Lab of Environmental Health and Engineering department for their teaching and instructions when I first joining the lab and on the way. They have been great mentors for me not only academically but also personally and great friends. Their helps and encouragement keep the project and me moving forward.

In the third, I would like to thank all members from Betenbaugh Lab, Bouwer Lab and Preheim Lab for their supports and friendships. They have truly made the experience here special for me. In addition, I would also like to thank Dr. Pavlo Bohutskyi from Pacific Northwest National Laboratory and Dr. Cristal Zuniga form University of California San Diego for their guidance on this project.

Last but not least, I would like to thank my parents and my entire family for everything they provided. Without their supports and belief for me, I will never be able to go this far and continue to my Ph.D. I will never be able to pay them back and I will do all I can to continually make them proud.

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LIST OF ABBREVIATIONS

S. elongatus cscB – *Synechococcus elongatus* PCC 7942, with cscB gene

E. coli W – *Escherichia coli* ATCC9637

LB medium – Lysogeny broth medium

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

EDTA – Ethylenediaminetetraacetic acid

INTRODUCTION

In nature, microorganisms do not level as axenic cultures, but interact with each other in complex networks. Lichen is a great example for such a network in nature, which is a symbiotic system consisting photosynthetic producers like algae or cyanobacteria and fungus.(Oksanen, 2006) Fundamentally speaking, co-culture is not very different from lichen but not limited to the paring of autotrophs and heterotrophs. Co-culture is a system consisting of two or more species of cells that interacts on some level.(Goers, Freemont, & Polizzi, 2014) Co-culture has been used as the tool in studying cells interactions, improving cultivation success, creating experiment model or simulating natural systems like in tissue engineering.(Goers et al., 2014) It has long been used in in industrial applications as mixed cultures as well, for example in the production of bioethanol form celluloses. (Energy, 2006)

Taking inspiration form pervious works in Betenbaugh labs involving synthetic lichens, an attempt is made to build a new co-culture system involving a photosynthetic producer and a partner that is capable consuming the organic metabolite and biomass produced by producer, like a natural lichen system. However, the goal of this study is beyond of building a stable synthetic lichens system but also to build a genome scale model for the synthetic lichen or co-culture systems and to use the system as a potential production system in future. Although there are well-known examples of photosynthesis, like *Nostoc* species, which was used in our labs previous studies, the polysaccharides they produce cannot be used as feedstock for industrial production species like *Escherichia coli*.(Paulsrud & LINDBLAD, 1998) Thus, the genetically engineered cyanobacteria strain, *Synechococcus elongatus* cscB, is chosen for its ability to produce and excrete sucrose, which can be used as feedstock for *Escherichia coli* ATCC 9637. (Ducat, Avelar-Rivas, Way, & Silver, 2012)

1.1 *Synechococcus elongatus* cscB

The wildtype *Synechococcus elongatus* PCC 7942 is a freshwater cyanobacterium strain and is the first cyanobacterium found that can consistently transform DNA in alien origin to itself. Hence, it comes with many reliable genetic tools for genetic engineering and producing consistent result. (Nordberg H et al., 2014) In addition to genetic engineering capability, the *Synechococcus elongatus* PCC 7942 can produce high amount of sucrose as a response to high salinity environment in order to balance the osmatic pressure across the cell membrane. (Ducat et al., 2012) In a study, regarding the photosynthetic efficiency of the *Synechococcus elongatus* PCC 7942, a group genetically engineered a *E. coli* sucrose permease gene, the cscB gene, into *Synechococcus elongatus* PCC 7942 thus produced the *Synechococcus elongatus* cscB stain, which will be used in this study for its ability to export sucrose from cellular space to medium.(Abramson, Kachel, Kramer, & Ducat, 2016)

Finally, *Synechococcus elongatus* PCC 7942 has its complete chromosome sequence and cellular plasmid sequence in archive, thus simplify the process of constructing a genome scale model for *Synechococcus elongatus* cscB. (Chen, Holtman, Magnuson, Youderian, & Golden, 2008)The *Synechococcus elongatus* cscB has also been used in some previous studies from our lab with great results when co-culture with fungus. (Li et al., 2017) Besides the advantages as a well-studied microorganism, has also be tested for large scale industrial productions, which increases the potential impacts of this study.(Diamond, Jun, Rubin, & Golden, 2015)

1.2 *Escherichia coli* ATCC 9637

Escherichia coli strains are among the most well-studied microorganisms in academic and industrial world.(Blount, 2015) Due to the simplicity of their genome, vast genetic tool kits and great adaptability in producing virous proteins, peptides, and other products, *Escherichia*

coli strains are frequently used as model organism in microbiology studies.(Vila et al., 2016) For the same reason, strains have also been widely used in industrial production of various chemical compounds and many pharmaceutical compounds and proteins.(Berry, 1996; Westers, Westers, & Quax, 2004)

As a member of this species, *Escherichia coli* ATCC 9637 does not only have a complete genome sequence in archive, but constructed genome models ready for use, which makes the construction of genome scale model for co-culture much easier. (Monk et al., 2013)

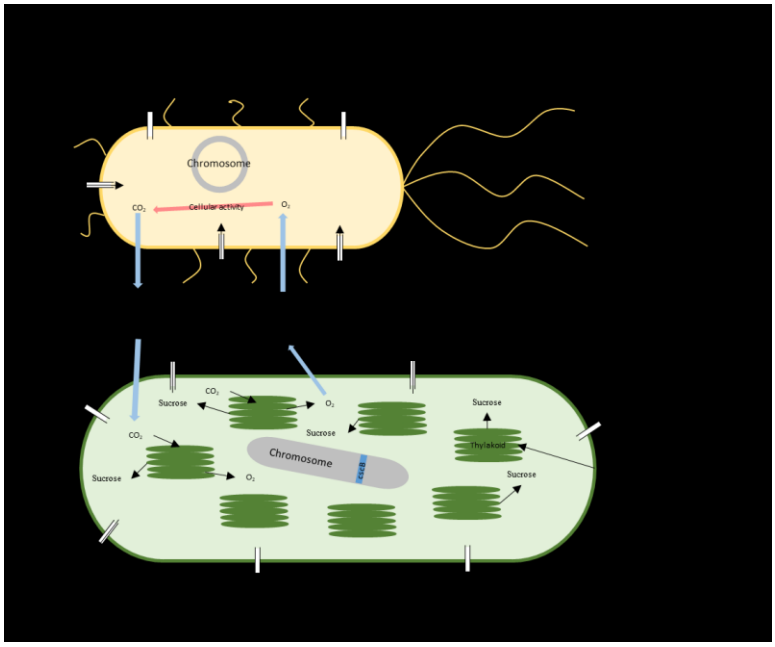


Figure 1. **Illustration of the expected interactions of the proposed co-culture system** (Abramson et al., 2016; Yu et al., 2015)

A co-culture system involving *Synechococcus elongatus* cscB and *Escherichia coli* ATCC 9637 may be very desirable as it could generate a low-cost production method by allowing *Escherichia coli* ATCC 9637 grows on the carbon source, sucrose, which is fixed by *Synechococcus elongatus* cscB from carbon dioxide. Such a system, containing two model microorganisms with great application potential in pharmaceutical and chemical production industries, may have significant impacts on industrial production method and economy.

MATERIALS AND METHODS

2.1 Strains and growth condition

The cyanobacteria strain *Synechococcus elongatus* cscB is kindly provided by Dr. Daniel Ducat form Michigan State University and is grown at 28 °C temperature in 100mL BG-11 medium with 3 g/L HEPES added and agitated with air containing additional 1% (v/v) CO_2 which sterilized by 0.2 μm filter (Corning PES syringe filter, 25mm), in 250 mL Erlenmeyer flask. The illumination condition is 100 μmol/m^2/s light intensity with 16:8 hours light/dark cycle, which is provide by cool-white fluorescent lamps. 100 mM NaCl is added into *S. elongatus* cscB culture, 24 to 48 hours before experiments.

The *Escherichia coli* strain ATCC 9637 is purchased from American Type Culture Collection and is grown at 28 °C temperature in 50 mL LB medium and agitated by stir bar in 125 mL Erlenmeyer flask. The knockout strains of *Escherichia coli* strain ATCC 9637, ΔarsB, ΔascF, Δglts, ΔlacZ, Δmak, ΔpflB, Δpgi and Δtpi are kindly provided by Australian Institute for Bioengineering and Nanotechnology. *E. coli* W and its knockouts are transferred from LB medium to BG-11 medium supplemented with 100mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 5 g/L sucrose for overnight culture (16 to 20 hours), before experiments. Due to the knockout of the tpi gene, 32.16 mM phosphate is also added for medium used to culture *E. coli* W Δtpi stock.

All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps.

The control medium used consists of BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG added. BG-11 medium is prepared by following the recipe: 1.5 g/L $NaNO_3$, 0.04 g/L $K_2HPO_4 \cdot 3H_2O$, 0.02g/L $NaCO_3$, 0.075 g/L $MgSO_4 \cdot 7H_2O$, 0.035g/L $CaCl_2$,

0.00024 g/L citric acid, 0.0004 g/L EDTA·$2H_2O$, 0.00024 g/L ferric ammonium citrate, and 1mL trace metal solution per liter. The trace metal solution includes 2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2 \cdot 4H_2O$, 0.222 g/L $ZnSO_4 \cdot 7H_2O$, 0.39 g/L $NaMoO_4 \cdot 2H_2O$, 0.079 g/L $CuSO_4 \cdot 5H_2O$, 49.4 mg/L and $Co(NO_3)_2 \cdot 6H_2O$.("BG-11 Medium for Blue Green Algae,") HEPES is added by using 300g/L stock solution with pH 8.9. Phosphate is added into medium by using a stock solution containing 150 g/L Na_2HPO_4 and 75 g/L KH_2PO_4 with pH 6.8. M9 medium used in experiments is prepared by following the recipe: 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, and 1 g/L NH_4Cl.("M9 Minimal Media Plates,") The LB medium is prepared with 20 g/L LB powder, purchased from Sigma-Aldrich™.

E. coli W supernatant is prepared by collecting liquid culture in control medium, with 3 g/L sucrose as carbon source and 32.16 mM phosphate, 8 to 12 hours after reaching stationary phase with 0.01 OD_{600} as initial cell density; centrifuged to collect supernatant and sterilized by passing through 0.2 μm filter. *S. elongatus* cscB supernatant are prepared by collecting liquid culture in control medum after 7 days of growth with 0.1 OD_{750} nm as initial cell density; centrifuged to collect supernatant and sterilized by passing through 0.2 μm filter.

2.2 Quantification of *S. elongatus* cscB and *E. coli* W

S. elongatus cscB growth is monitored by measuring optical density at 750 nm with Ultrospec 3100 pro for both axenic culture and coculture. The cell density has following correlation with OD_{750}:

$$\frac{Cells}{mL} = 1.84 \times 10^8 \times OD_{750} \qquad R^2 = 0.967$$

The cell counts used for generating this correlation are measured by BD FACSCalibur analytical flow cytometry. For axenic culture, this equation is applied directly; for co-culture, *E. coli* W's

effect on optical density is subtracted by using the following correlation based on *E. coli* W cell density:

$$Theroretical\ OD_{750} = 5.53 \times 10^{-10} \times \frac{Cells}{mL} \qquad R^2 = 0.999$$

E. coli W growth in axenic culture is monitored by measuring optical density at 600 nm with Ultrospec 3100 pro. The cell density has following correlation with OD_{600}:

$$\frac{Cells}{mL} = 1.23 \times 10^9 \times OD_{600} \qquad R^2 = 0.999$$

E. coli W growth in coculture is monitored by diluting culture liquid to 1 to 2 *E. coli* W cells per μL then plate on LB agar plate for cell counting. The same method is also used to obtain the cell density and OD_{600}/ OD_{750} relationships mentioned above.

2.3 Measurements and analysis

2.3.1 Sucrose measurement

The sucrose concentration is measured by using YSI 2700 SELECT biochemistry analyzer with YSI 2703 Sucrose Membrane Kit and YSI 2780 Sucrose Standard [5.00 g/L].

2.3.2 pH measurement

The pH of samples is measured by using Orion Star A211 pH meter from Thermo Scientific with Orion 9110DJWP double junction pH electrode from Thermo scientific. The set-up is standardized with pH 4, 7, and 10 standard solution prior to sample measuring.

2.3.3 Ammonium measurement

The free ammonium concentration is measured by combining 100 μL samples ($[NH_3]$ < 1 mM) with 100 μL assay regent, which is made by adding 5 mL of ethanol with 270 mg

phthalic dicarboxaldehyde dissolved in it and 50 μL of β-mercaptoethanol to 100 mL of 0.2 M pH =7.3 phosphate buffer. Mix the sample and reagent then react in room temperature in darkness for 30 min. Molecular Devices SPECTRAmax GEMINIXPS fluorescence spectrophotometer is used to analyze the samples with 410 nm excitation wavelength and 472 nm emission wavelength. Standard curve is prepared by measuring solution with known concentration of NH_4Cl solutions. Effective range is 0 to 1 mM NH_4^+. (Barney, Eberhart, Ohlert, Knutson, & Plunkett, 2015)

2.3.4 Phosphate measurement

The free phosphate or reactive phosphorus concentration is measured by ascorbic acid method. Mix 10 mL diluted sample with 0.2 mL 10% (w/v) ascorbic acid, then add 0.4 mL of reagent and mix again. Allow the reaction to proceed for 15 min at room temperature then measure the absorbance at 700 nm with Ultrospec 3100 pro. The reagent is prepared by adding 100 mL 130 g/L ammonium molybdate solution and 100 mL 3.5 g/L antimony potassium tartrate solution into 300 mL 50% (v/v) sulfuric acid solution. Standard curve is prepared by measuring solution with known concentrations of KH_2PO_4 solutions. Effective range is 0.01-0.6 mg/L phosphorus. (Rice, Baird, & Eaton, 1999)

RESULTS

3.1 Medium modification for *E. coli* W growth

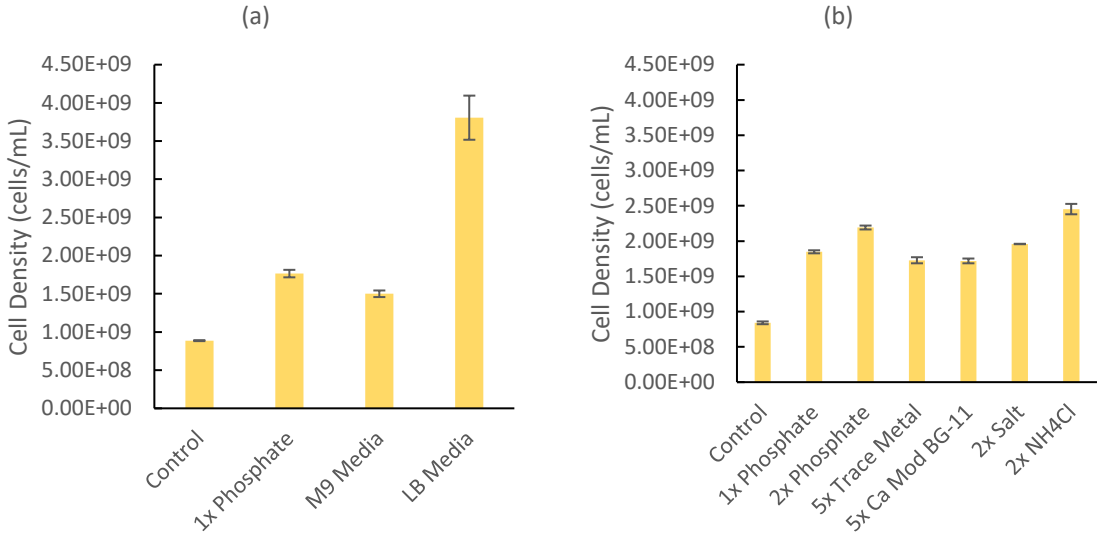


***Figure 2*. *E. coli* W growth under various condition.**
(a) *E. coli* W growth in different medium. (b) Medium Optimization for *E. coli* W growth. "Control" is the medium consisting BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG. "1x Phosphate" is "Control" medium with 64.31 mM Phosphate. For (b) all medium contains 64.31 mM phosphate except "Control" and "Phosphate", which contains 0.175 mM and 128.62 mM respectively. "Salt" includes $NaNO_3$, K_2HPO_4, Na_2CO_3 and $MgSO_4$. "Trace metal" includes H_3BO_3, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $NaMoO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, and $Co(NO_3)_2{\cdot}6H_2O$ All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 4 g/L sucrose in medium and all data are collected at 22 hours after inoculation. Cell growth is monitored by measuring optical density at 600 nm.

To evaluate the possibility of building a co-culture system with *S. elongatus* cscB and *E. coli* W, we first evaluate the growth of *E. coli* W in control medium with sucrose as the primary carbon source. As Fig 2(a) shown, *E. coli* W grows poorly in the control even comparing with minimal medium M9, reaching only about 60% cell density in of M9 medium. However, with phosphate supplement at the same concentration as M9 medium, 64.31 mM, the *E. coli* W can reach 117 % cell density of in M9 medium. To further optimize the medium, different components in control medium are varied. Varying salts and trace metal concentration in medium show no improvement in growth. Although, doubling phosphate and ammonium concentration show 18% and 32% cell density increase respectively, the increases are not to

scale with the addition of extra materials. Finally, we test the pH of the control medium, control medium with M9 level phosphate ("1x Phosphate" in figures), control medium with 2x phosphate supplement and control medium with 2x ammonium supplement. The results are 4.92 ± 0.006, 5.29 ± 0.002, 5.26 ± 0.015 and 5.26 ± 0.016 respectively.

3.2 Effect of Phosphate on *S. elongatus* cscB and *E. coli* W growth

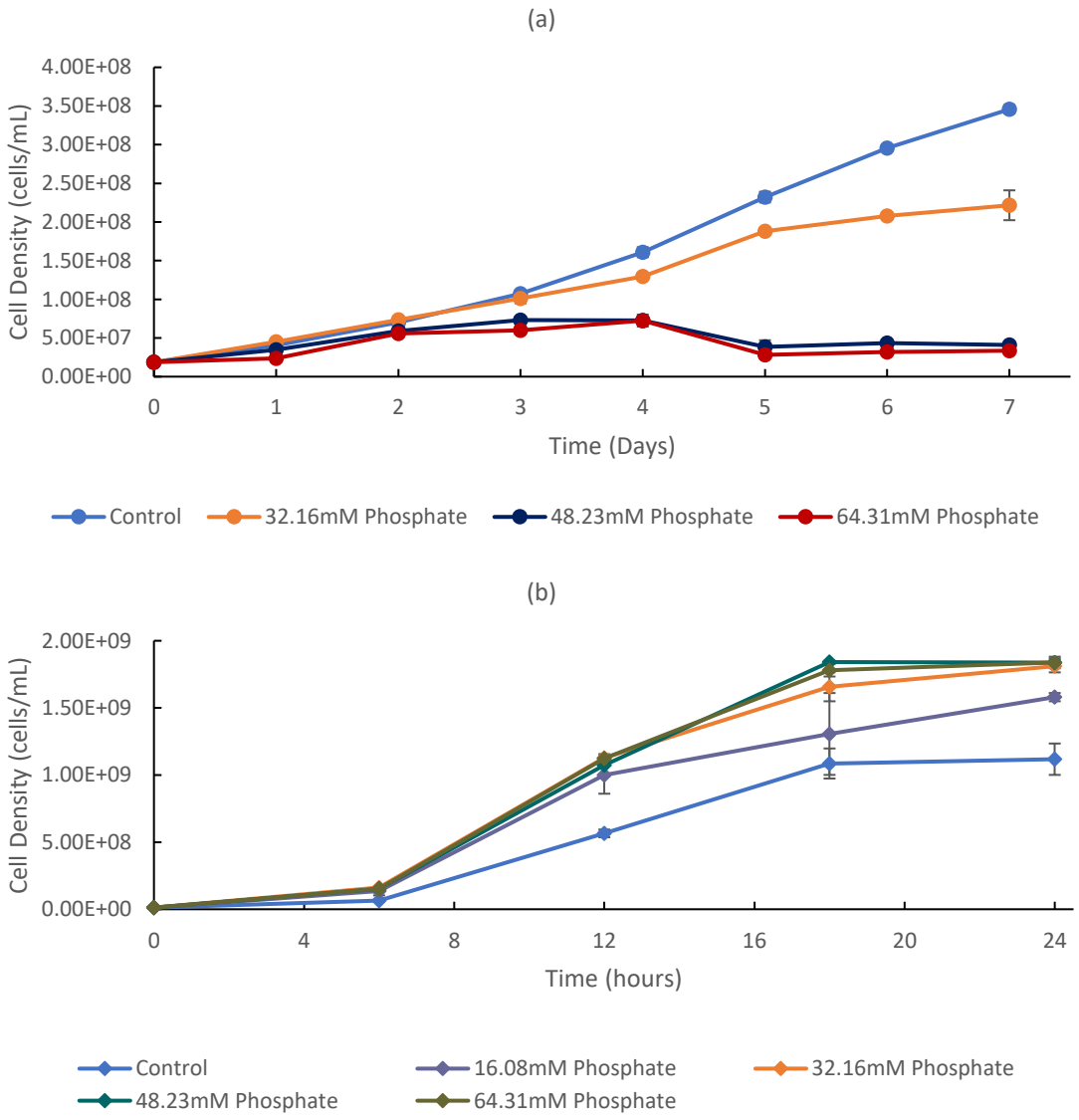


Figure 3. **Effects of Phosphate on cell growth.**
(a) Effect on *S. elongatus* cscB growth. (b) Effect on *E. coli* W growth. Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG, with different phosphate concentrations as indicated. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. For *E. coli* W experiments, 4

g/L sucrose are added into medium. *S. elongatus* cscB and *E. coli* W growths are monitored by measuring optical density at 600 nm and 750 nm respectively.

Since some cyanobacteria shows low tolerance to phosphate (Ernst, Deicher, Herman, & Wollenzien, 2005), the effect of phosphate on *S. elongatus* cscB growth is tested. As shown in Fig. 3(a), both the growth rate and the maximum cell density are negatively impacted by phosphate addition, which slows the growth rate and severely impact max cell density. At 48.23 mM phosphate and above, *S. elongatus* cscB reaches maximum cell density at day 3 and start dying at day 4. At 32.16 mM phosphate, *S. elongatus* cscB although grows slowly but remains to grow until day 7. Due to the negative impact of phosphate on *S. elongatus* cscB, the effect of phosphate on *E. coli* W growth is inspected as well, shown in Fig. 3(b). Although phosphate is necessary for improving the growth of *E. coli* W in control, 32.16 mM and above concentration of phosphate showed similar growth patterns. Thus, 32.16 mM phosphate is chosen to be the concentration in coculture medium, as it shows neglectable impact on *E. coli* W growth and can maintain relative acceptable growth rate and maximum cell density of *S. elongatus* cscB.

3.3 Effect of *S. elongatus* cscB and *E. coli* W supernatants on growth of each other

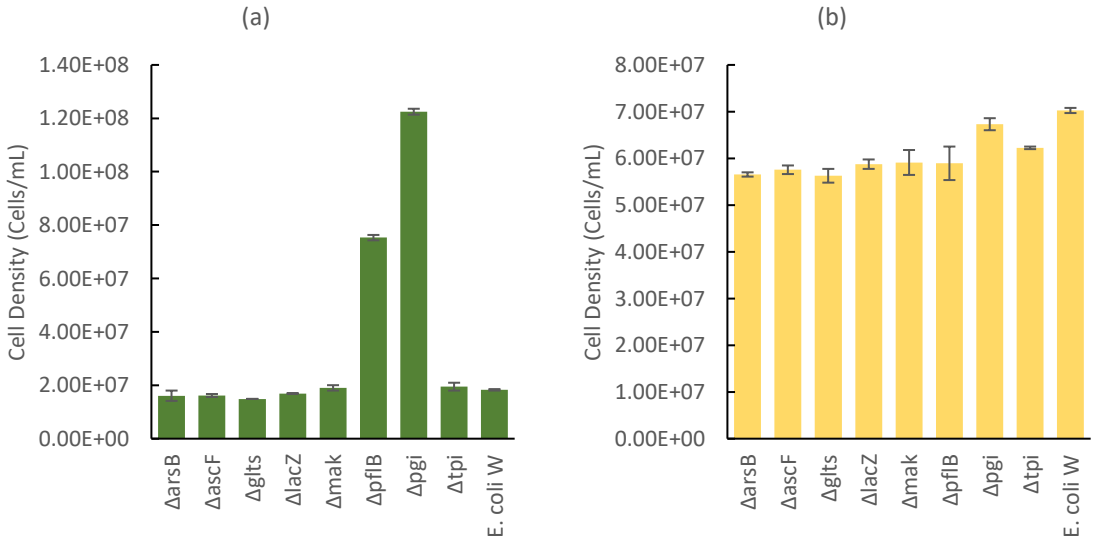


Figure 4. **Effects of supernatants on growth of *S. elongatus* cscB and *E. coli* W.**

(a) *S. elongatus* cscB growth in *E. coli* W and its knockouts supernatants. (b) *E. coli* W and its knockouts growths in *S. elongatus* cscB supernatants. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *S. elongatus* cscB and *E. coli* W growths are monitored by measuring optical density at 600 nm and 750 nm respectively. *S. elongatus* cscB are cultured for 3 to 7 days depending on when the cells reach maximum cell density or die. *E. coli W* is cultured for 24 to 48 hours depending on when the cells reach maximum cell density or die. *E. coli* W supernatant is prepared by collecting liquid culture, in BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG, 32.16 mM phosphate, and 3 g/L sucrose as carbon source, 8 to 12 hours after reaching stationary phase. *S. elongatus* cscB supernatant are prepared by collecting liquid culture in BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG, after 7 days of growth and phosphate is added when inoculate the supernatant with *E. coli.* Both supernatants are sterilized by passing through 0.2 µm filter.

Gene	Enzyme	Source
lacZ	Beta-galactosidase	https://www.uniprot.org/uniprot/P00722
mak	Fructokinase	https://www.uniprot.org/uniprot/P23917
pflB	Pyruvate formate lyase I	https://www.uniprot.org/uniprot/E0IZD9
ascF	fused cellobiose/arbutin/salicin-specific PTS enzymes	http://ecoliwiki.net/colipedia/index.php/ascF:Gene_Product(s)
arsB	Arsenical pump membrane protein	https://www.uniprot.org/uniprot/P0AB93
gltS	Sodium/glutamate symporter	https://www.uniprot.org/uniprot/P0AER8
tpiA	Triosephosphate isomerase	https://www.uniprot.org/uniprot/P0A858
pgi	Glucose 6-phosphate isomerase	https://www.uniprot.org/uniprot/P0A6T1

Table 1. **Enzymes or protein structures corresponding to knockout genes**

To further test the feasibility and select suitable knockouts for co-culture test, *S. elongatus* cscB and *E. coli* W is inoculated to grow on each other's supernatant. For *S. elongatus* cscB supernatant, phosphate is added during inoculation of *E. coli* W and knockouts. *E. coli* W's and its knockouts' supernatants show considerable toxicity to *S. elongatus* cscB. With $(1.84 \pm 0.056) \times 10^7$ cells/mL initial cell density, *S. elongatus* cscB shows no growth in all supernatants collect except ΔpflB and Δpgi. In addition, *S. elongatus* cscB cells die out 2 days after inoculation in supernatants of ΔarsB and Δglts, 6 days after inoculation in ΔpflB and Δpgi, and 4

days after inoculation in the rest of supernatants. On the other hand, *E. coli* W and its knockouts showed good growth in all supernatant with 32.16 mM phosphate supplement, as the sucrose level in collected *S. elongatus* cscB supernatant is 538 ± 2 mg/L. Judging from the data, ΔpflB and Δpgi are chosen to be tested and *E. coli* W wild type serves as the control in co-culture

3.4 *E. coli* W inoculation timing

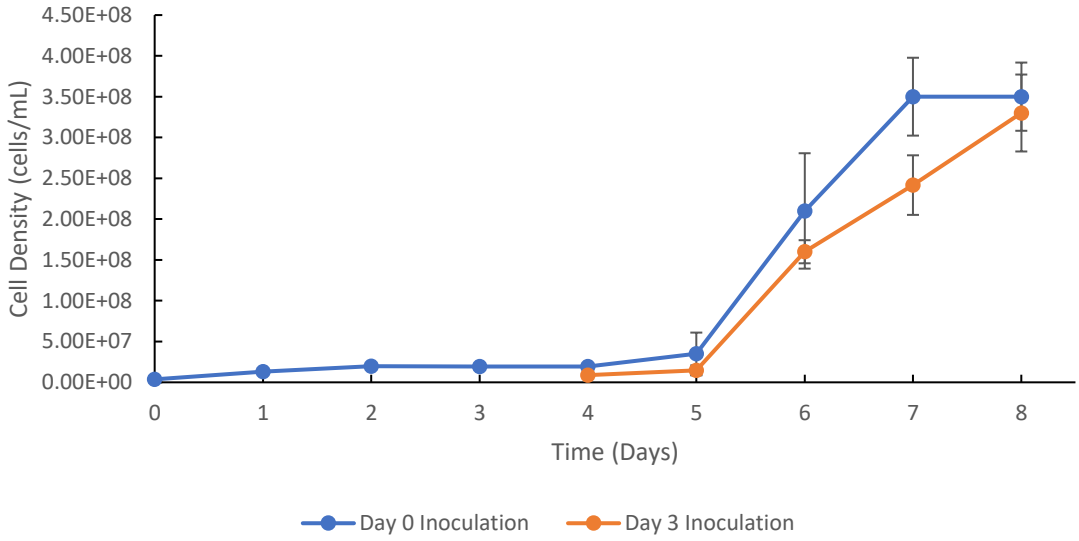


Figure 5. **Effects of *E. coli* W inoculation timing on its growth.**
Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, 3 g/L HEPES and 1 mM IPTG, with 32.16 mM phosphate. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W growths are monitored by plating diluted samples on LB agar plates.

S. elongatus cscB produces no or very low amount of sucrose in first 4 days after inoculation, as shown in later figures from section 3.5, 3.6 and 3.7, thus the timing of inoculation is investigated. Based on the result shown in Fig 5, the inoculation timing does not impact the *E. coli* W growth strongly. However, due to concern with cell viability after 3 days in co-culture system, the inoculation timing is set at 3 days after *S. elongatus* cscB inoculation, as there are cell density decreases from day 0 to day 3 in some trails.

3.5 pH control's impacts on axenic culture and co-culture

HEPES buffer is added in the medium to provide pH control, since the cscB gene engineered into *S. elongatus* cscB codes a cotransporter of H^+ and sucrose, which moves the sucrose across the pH gradient. (Ducat et al., 2012) Thus, a basic medium environment should be favorable for sucrose production and exportition. So, the impact of pH control is studied.

3.5.1 pH control's impact on *S. elongatus* cscB growth

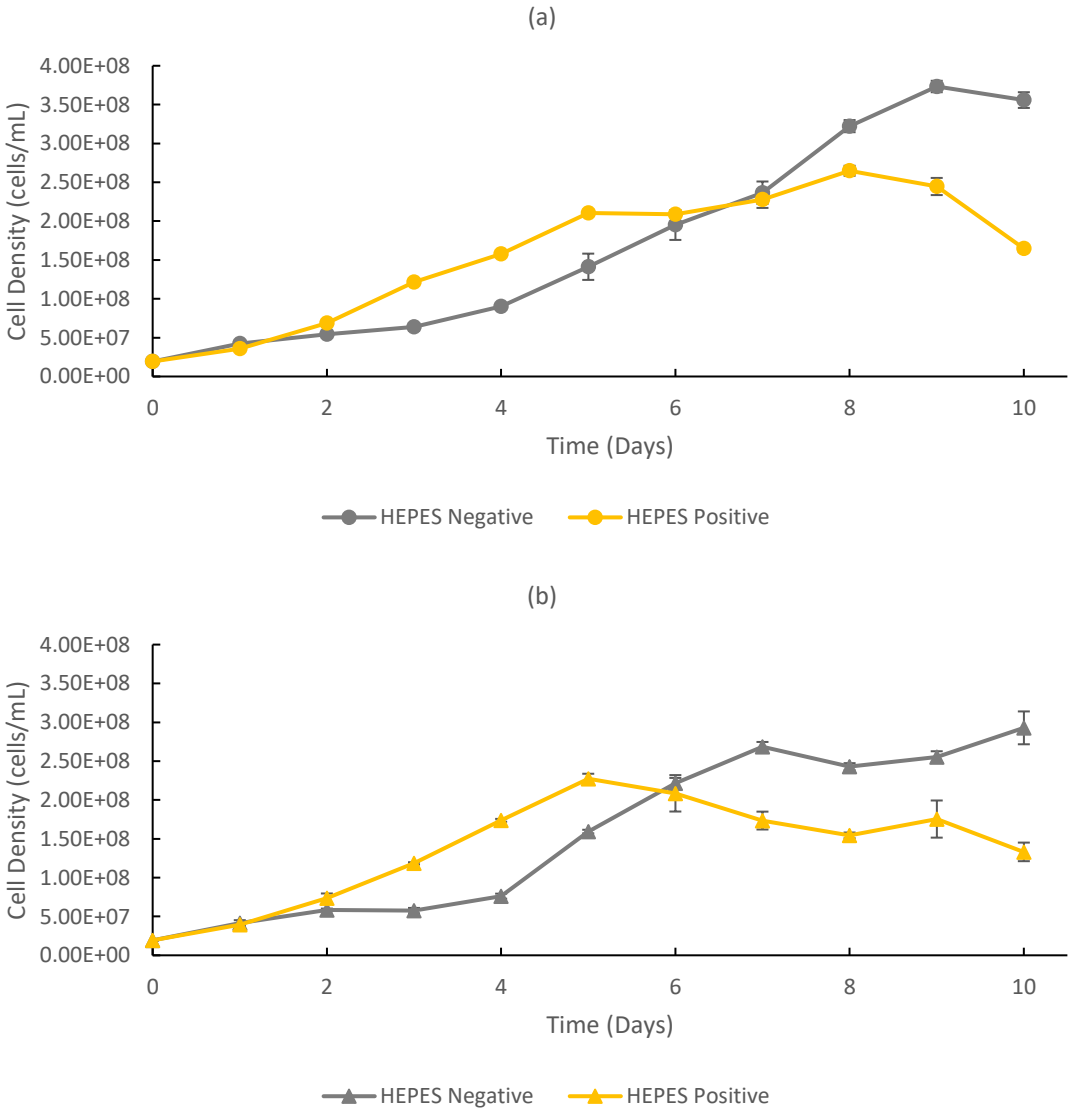


Figure 6. **pH control's effects on *S. elongatus* cscB growth.**

(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG, with 32.16 mM phosphate added at day 3. HEPES Negative contains no HEPES and HEPES positive contains 3 g/L HEPES. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *S. elongatus* cscB growths are monitored by measuring optical density at 750 nm. *E. coli* W is inoculated into co-culture system at day 3.

From Fig. 6, the *S. elongatus* cscB grows lower as excepted initially but there are significant increases in growth rate after day 4, in both axenic culture and co-culture, even surpassing the growth rate and maximum cell density despite both containing phosphate. Comparing the maximum cell density during the trail, *S. elongatus* cscB cell density of HEPES negative axenic culture achieved 87.5% cell density of the control axenic culture which is phosphate negative and contains HEPES for pH control. The HEPES positive culture only achieves 65.1% cell density of maximum control axenic culture, which happens at day 8 and then cell density gradually decreases, indicating culture entering death phase.

3.5.2 pH control's impact on *E. coli* W growth in co-culture

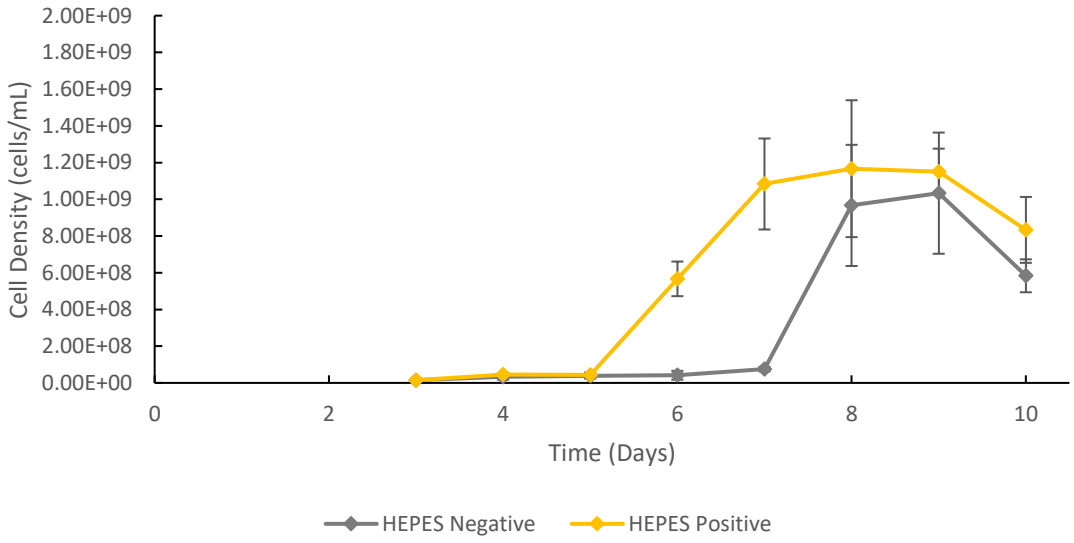


Figure 7. **pH control's effects on *E. coli* W growth in co-culture.**
Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG, with 32.16 mM phosphate added at day 3. HEPES Negative contains no HEPES and HEPES positive contains 3 g/L HEPES. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. *E. coli* W growths are monitored by plating diluted samples on LB agar plate.

As shown in Fig. 7, the *E. coli* W does not show sign of significant growth until day 8 in HEPES negative medium, comparing with day 5 in medium containing HEPES. The maximum cell density is also lower in HEPES negative medium than in HEPES positive medium, but not very significantly. Consider all, indicates that pH buffering has limited impact on *E. coli* W growth potential but prolongs the lag phase and possibly makes it more difficult for *E. coli* W to adjust for co-culture medium.

3.5.3 pH control's impact on sucrose production

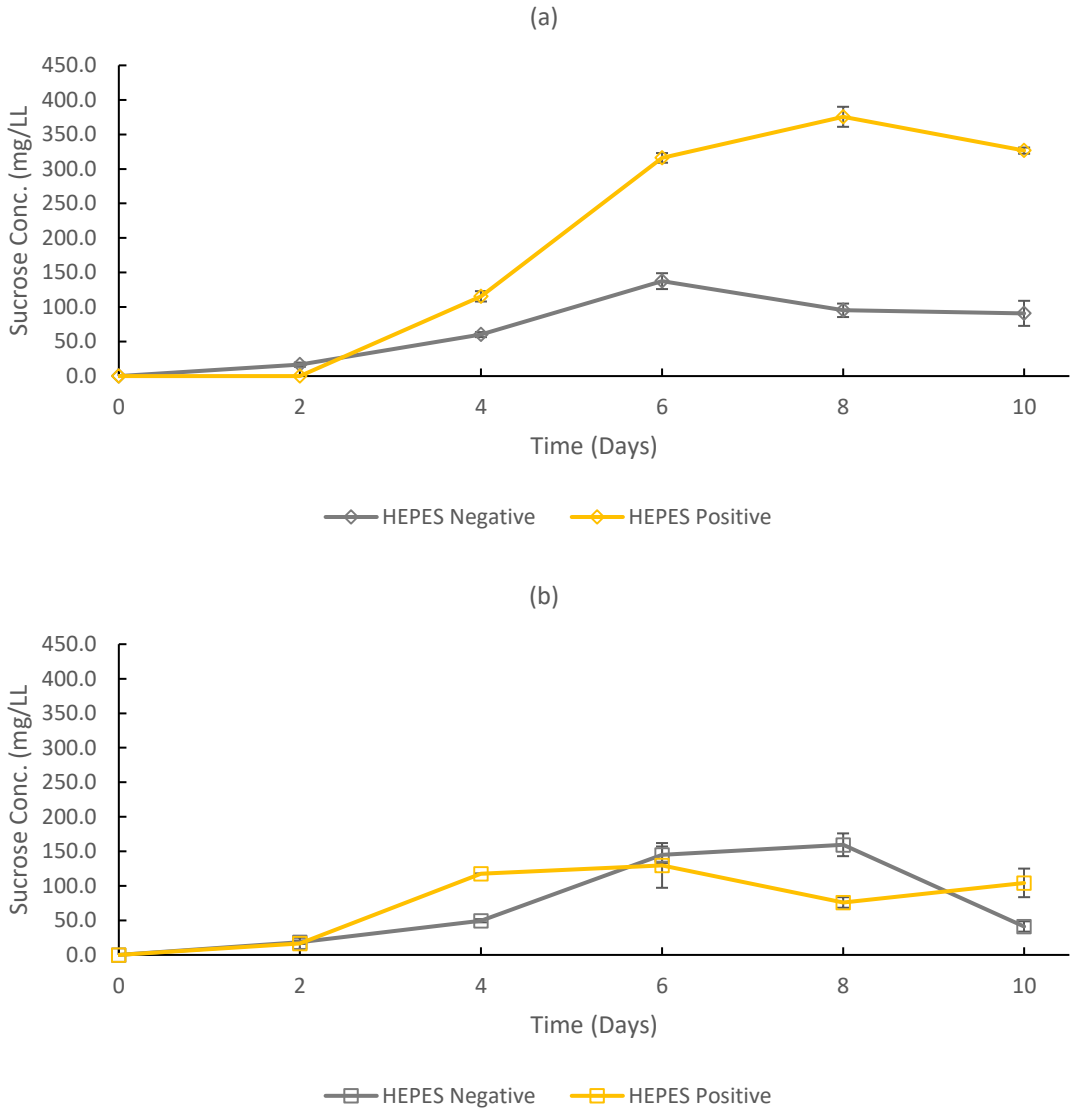


Figure 8. **pH control's effects on sucrose production.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG, with 32.16 mM phosphate added at day 3. HEPES Negative contains no HEPES and HEPES positive contains 3 g/L HEPES. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. Sucrose concentrations are measured by YSI 2700 SELECT.

The pH control ability plays a very important role in sucrose production as expected, shown in Fig. 8(a). The maximum sucrose concentration in HEPES negative axenic culture only reaches 36.7% of the HEPES positive axenic culture, indicating a significantly lower sucrose production and/or primarily exportation capability. However, the sucrose concentration in both coculture systems are very similar to each other. Considering the maximum *E. coli* W cell density is similar in both co-culture systems, the sucrose production and/or exportation rate may be much higher in co-culture than in axenic culture.

3.5.4 pH control's impact on pH

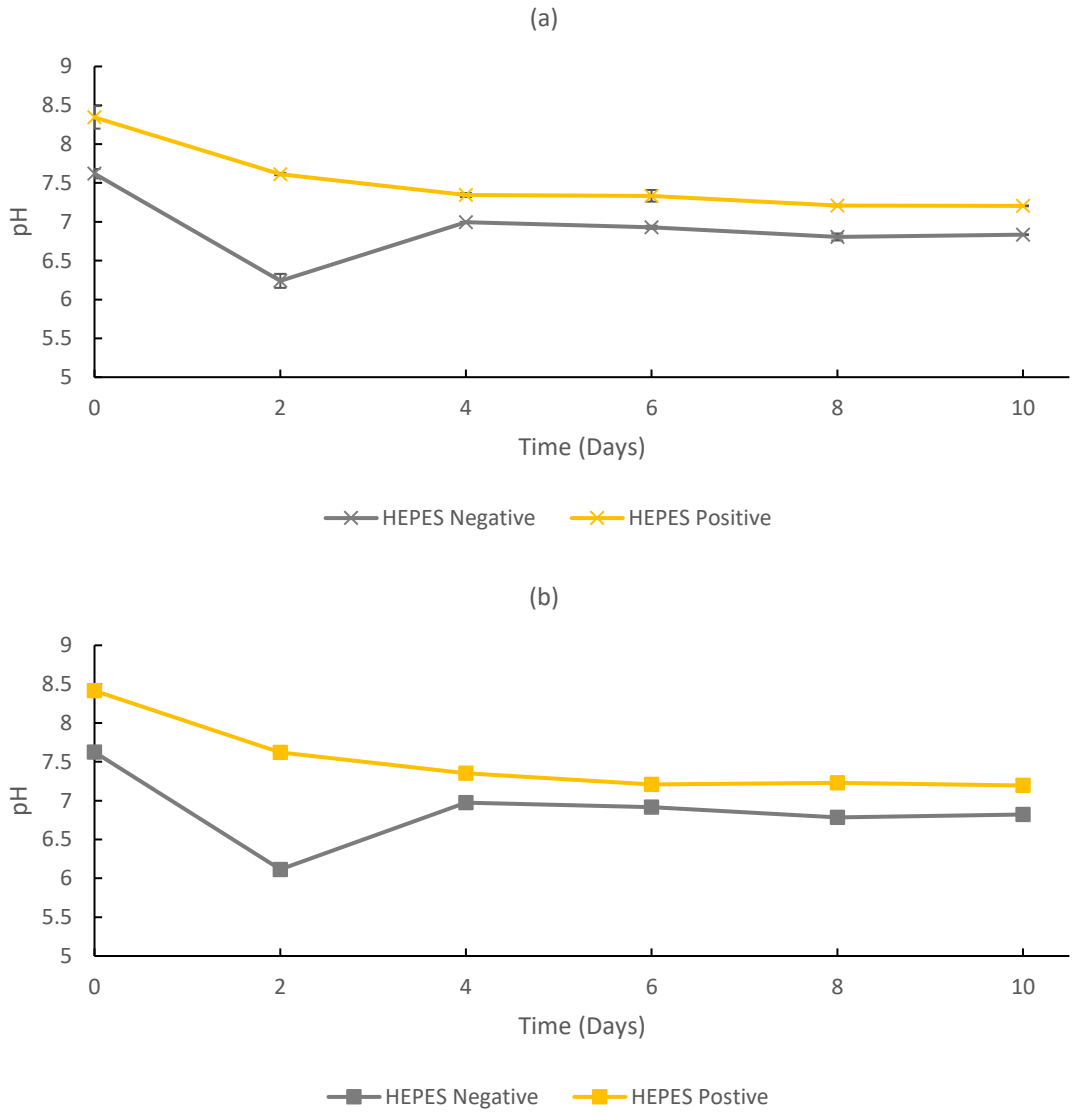


Figure 9. **pH control's effects on pH.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Medium used is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG, with 32.16 mM phosphate added at day 3. HEPES Negative contains no HEPES and HEPES positive contains 3 g/L HEPES. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. pH is measured by Orion Star A211 pH meter from Thermo Scientific with Orion 9110DJWP double junction pH electrode from Thermo scientific.

The pH of HEPES negative cultures acidify much quicker than HEPES positive cultures. However, after phosphate addition, the pH quickly rises back. After day 4, pH stabilizes in all tested conditions with neglectable fluctuations. It suggests that either a metabolic change in *S.*

elongatus cscB or more likely the phosphate solution added can also serve as pH buffer in the systems, which is supported by the fact that the prepared phosphate solution has a pH around 6.8, which is similar to stabilized pH in HEPES negative groups.

3.6 Effects of Phosphate addition and time of addition

Since the inoculation time of *E. coli* W is set at day 3, the effect of adding phosphate at day 0 and day 3 need to be studied, as phosphate impacts the growth of both *S. elongatus* cscB and *E. coli* W significantly.

3.6.1 Effect of Phosphate addition on *S. elongatus* cscB growth

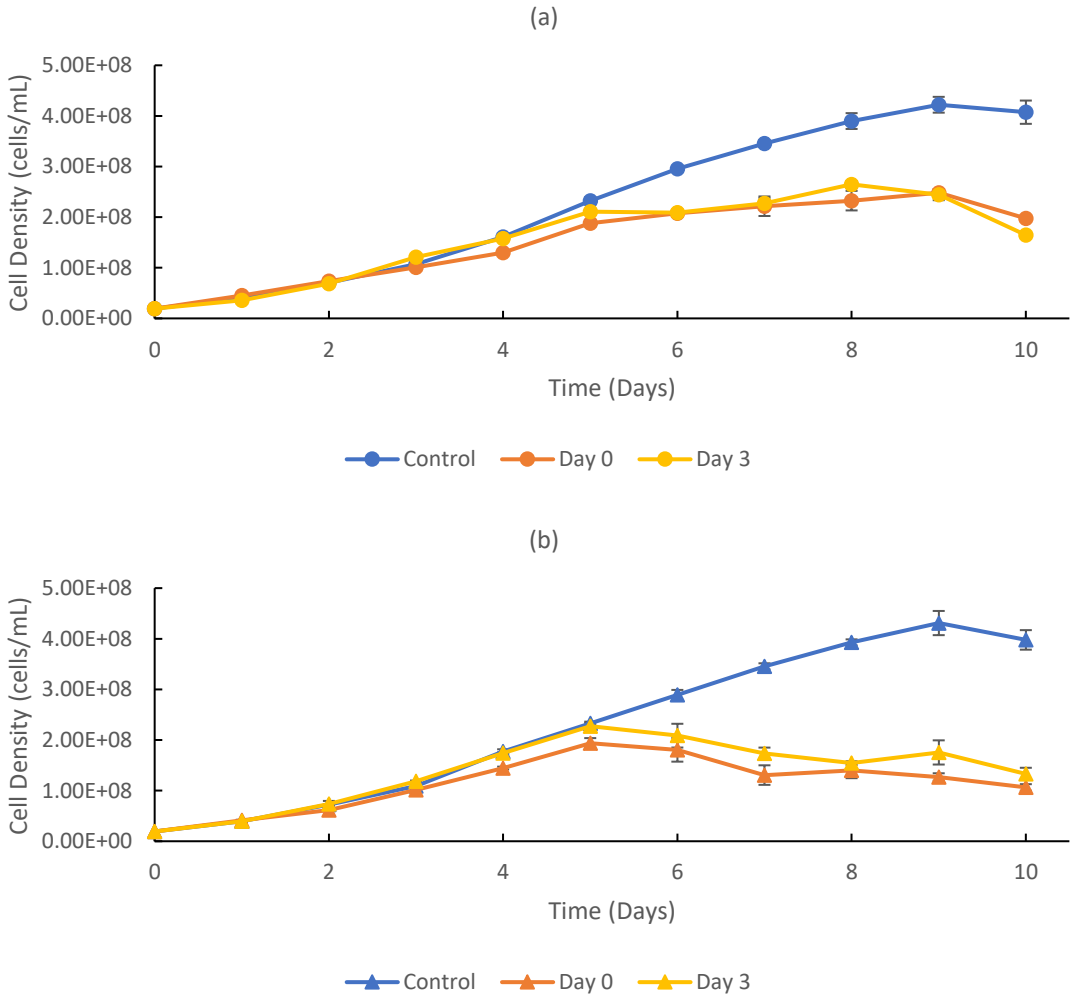


Figure 10. **Effects of phosphate addition timing on *S. elongatus* cscB.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. *S. elongatus* cscB growths are monitored by measuring optical density at 750 nm.

The phosphate addition shows negative impact on *S. elongatus* cscB growth as previously proved and the timing of phosphate addition shows no significant effects on *S. elongatus* cscB growth as there is no statistical difference in growth between day 0 addition culture and day 3 addition, in both axenic culture and co-culture.

3.6.2 Effect of Phosphate addition on *E. coli* W growth in co-culture

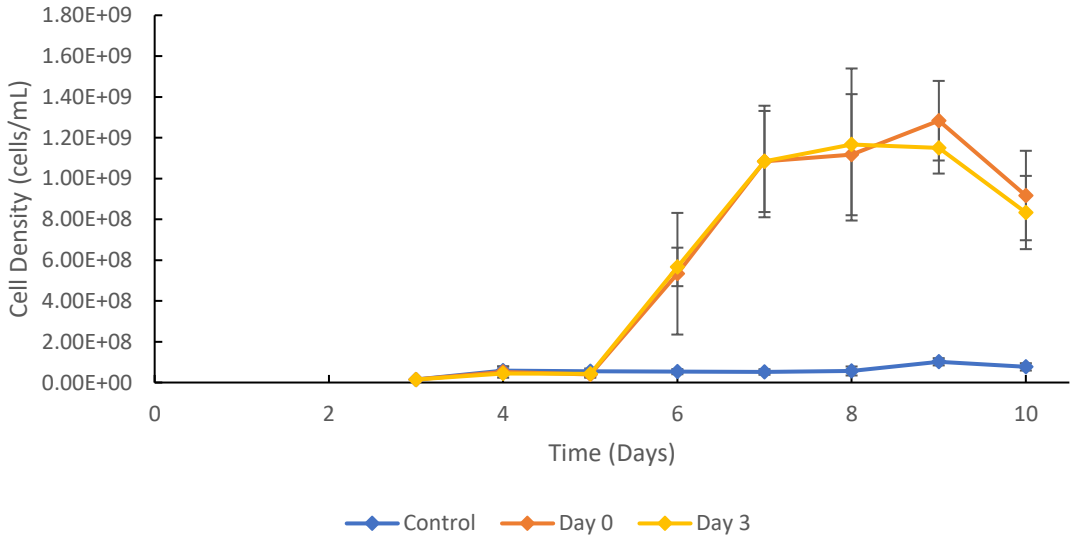


Figure 11. **Effects of phosphate addition on *E. coli* W growth in co-culture.**
Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. *E. coli* W growths are monitored by plating diluted samples on LB agar plate.

The phosphate addition shows positive impact on *E. coli* W growth as previously proved and the timing of phosphate addition shows not significant effects on *E. coli* W growth as there is no statistical different between Day 0 addition culture and Day 3 addition culture.

3.6.3 Effect of Phosphate addition timing on sucrose production

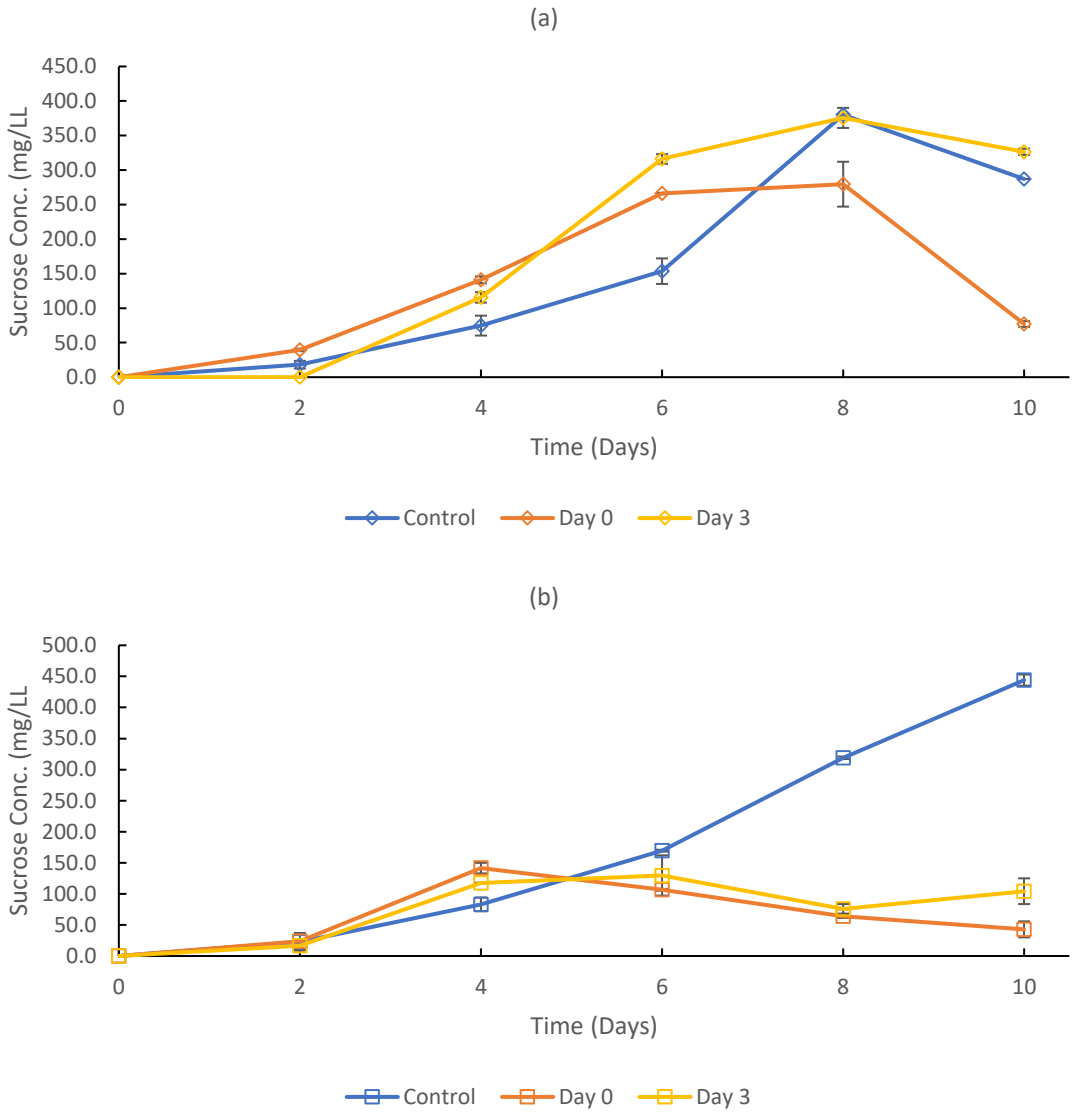


Figure 12. **Effects of phosphate on sucrose production.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. Sucrose concentrations are measured by YSI 2700 SELECT.

Based on the *S. elongatus* cscB axenic culture results, in Fig. 12(a), the phosphate increases the production rate of sucrose although not impacting the maximum sucrose accumulation in medium. The high concentration of sucrose in co-culture in control medium, Fig. 12(b), is likely the result of the low *E. coli* W growth, as shown in Fig. 11.

3.6.4 Effect of Phosphate addition timing on pH

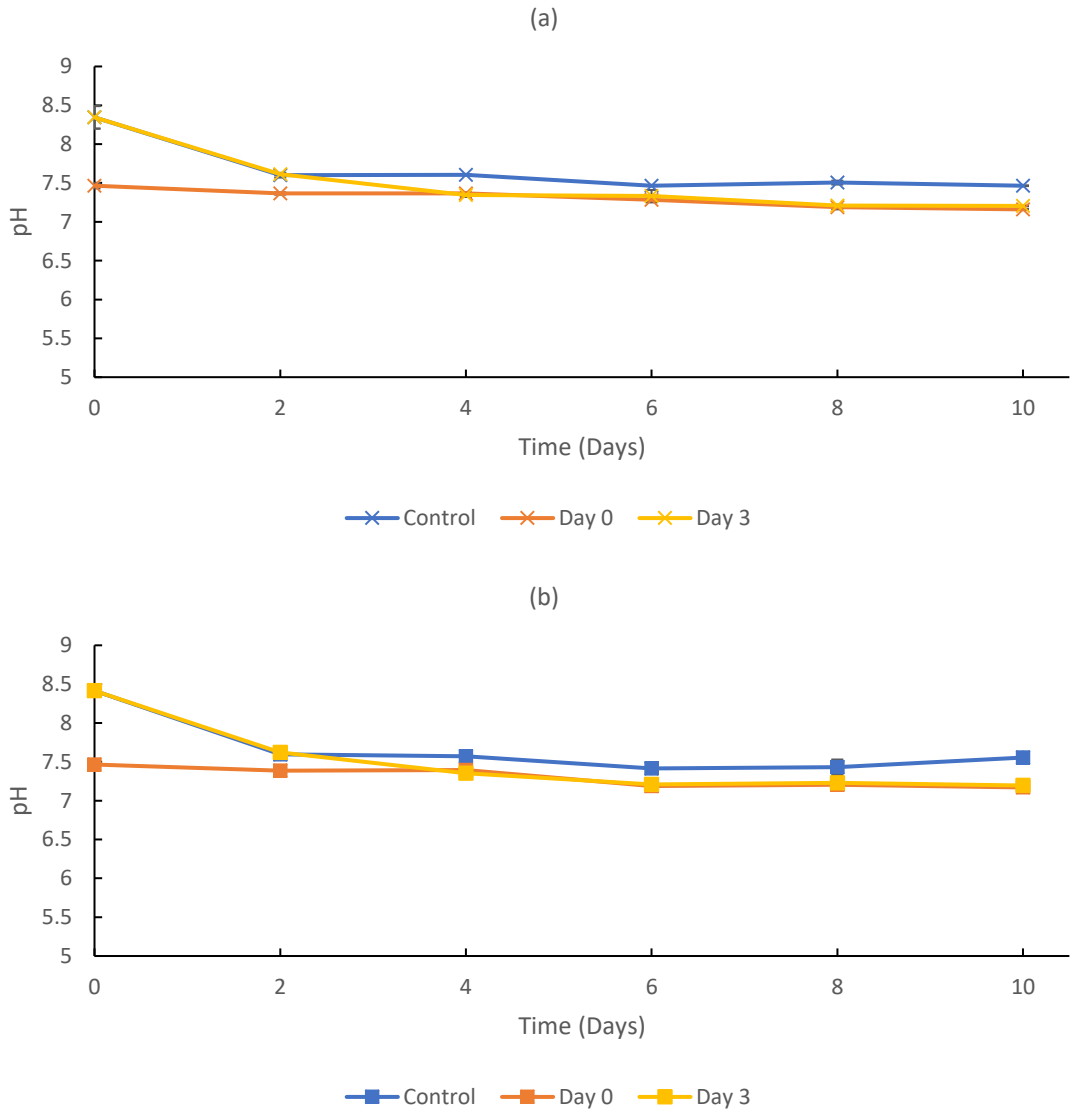


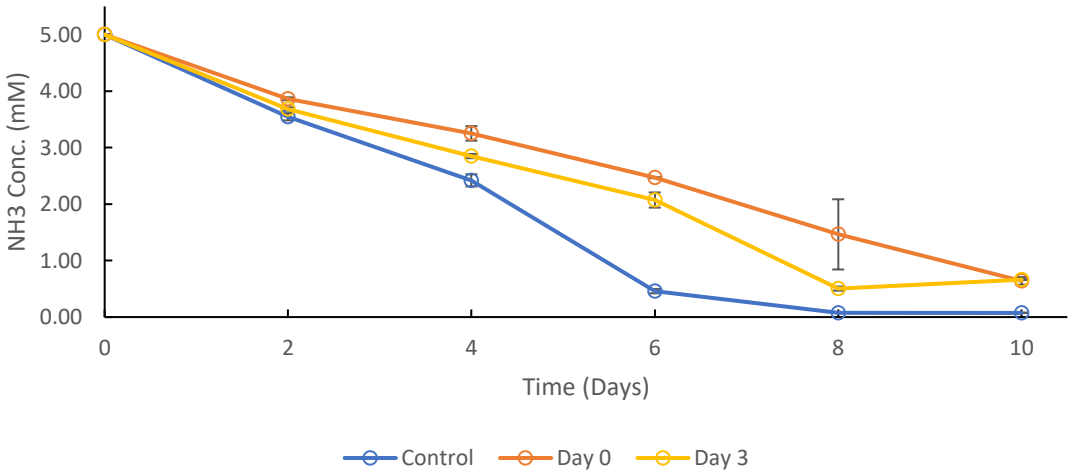
Figure 13. **Effects of phosphate effects on pH.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. pH is measured by Orion Star A211 pH meter from Thermo Scientific with Orion 9110DJWP double junction pH electrode from Thermo scientific.

As shown in Fig. 13, the two phosphate addition strategies results in almost identical pH profile starting from day 4, after the *E. coli* W inoculation, in both axenic culture and co-culture situation. However, the phosphate addition results in lower final pH of the medium. HEPES

buffer fails to control the pH once phosphate solution is introduced, as shown by decrease of pH form day 2 to day 4 in day 3 phosphate addition trials and the lower initial pH in day 0 addition trails. Except the day 3 phosphate addition trails, other trials' pH become stable after day 2. It is especially important that pHs in phosphate added co-culture trails stabilize at around 7.4 despite relative high *E. coli* W growth, which in *E. coli* W axenic culture stabilize at around pH 5.3 in stationary phase, as mentioned in section 3.1.

3.6.5 Effect of Phosphate addition timing on ammonium consumption

(a)



(b)

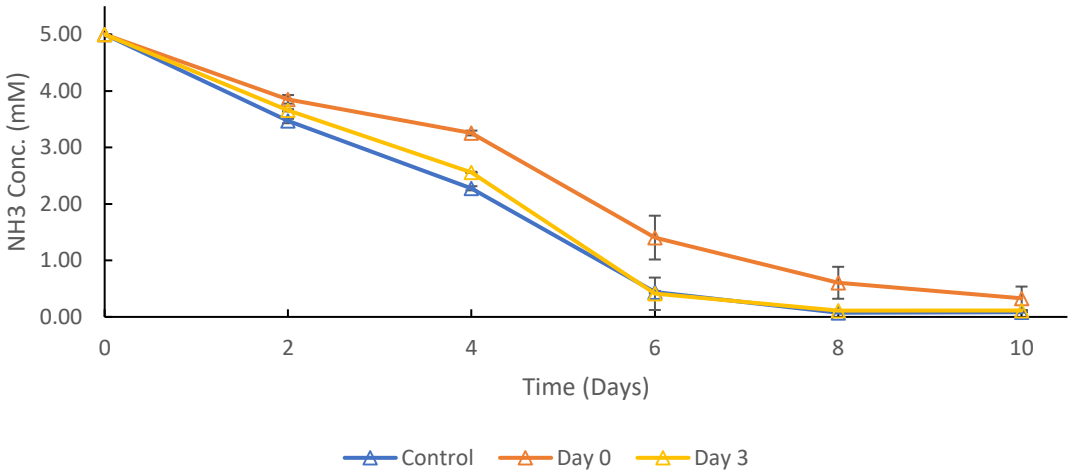


Figure 14. **Effects of phosphate on ammonium consumption.**
(a) *S. elongatus* cscB axenic cultures. (b) Co-culture systems. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimtal groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. The ammonium concentration is measured by using a reagent assay containing 5 mL of ethanol, 270 mg phthalic dicarboxaldehyde, and 50 μL of β-mercaptoethanol in 100 mL of 0.2 M phosphate buffer with a pH =7.3.

It has been suggested that ammonium addition in medium can help the growth of *S. elongatus* cscB (Ducat et al., 2012), and ammonium is the sole nitrogen source in M9 medium for *E. coli*. Thus, the utilization of ammonium in medium is studied. As shown in Fig. 14, ammonium is depleted in all scenarios. In Fig. 14(a), the axenic cultures of *S. elongatus* cscB with phosphate addition shows slower ammonium uptake rate than without phosphate addition which is most like the result of slower *S. elongatus* cscB growth. In co-culture trails, the trend holds, despite *E. coli* W growth.

However, it is worth noting that it has been observed in other studies, which is not in the scope of this thesis, that the *S. elongatus* cscB is capable of growing in ammonium negative medium or continue to grow beyond day 10 for another 5 to 7 days, when the ammonium is most likely depleted already.

3.7 Co-culture's impacts

The impacts that *S. elongatus* cscB and *E. coli* W have on each other are essential for building a successful co-culture system. Hence, many aspects of the co-culture system are studied in this section, from growth rates to nutrients consumption and pH change, etc.

3.7.1 Co-culture's impacts on *S. elongatus* cscB growth

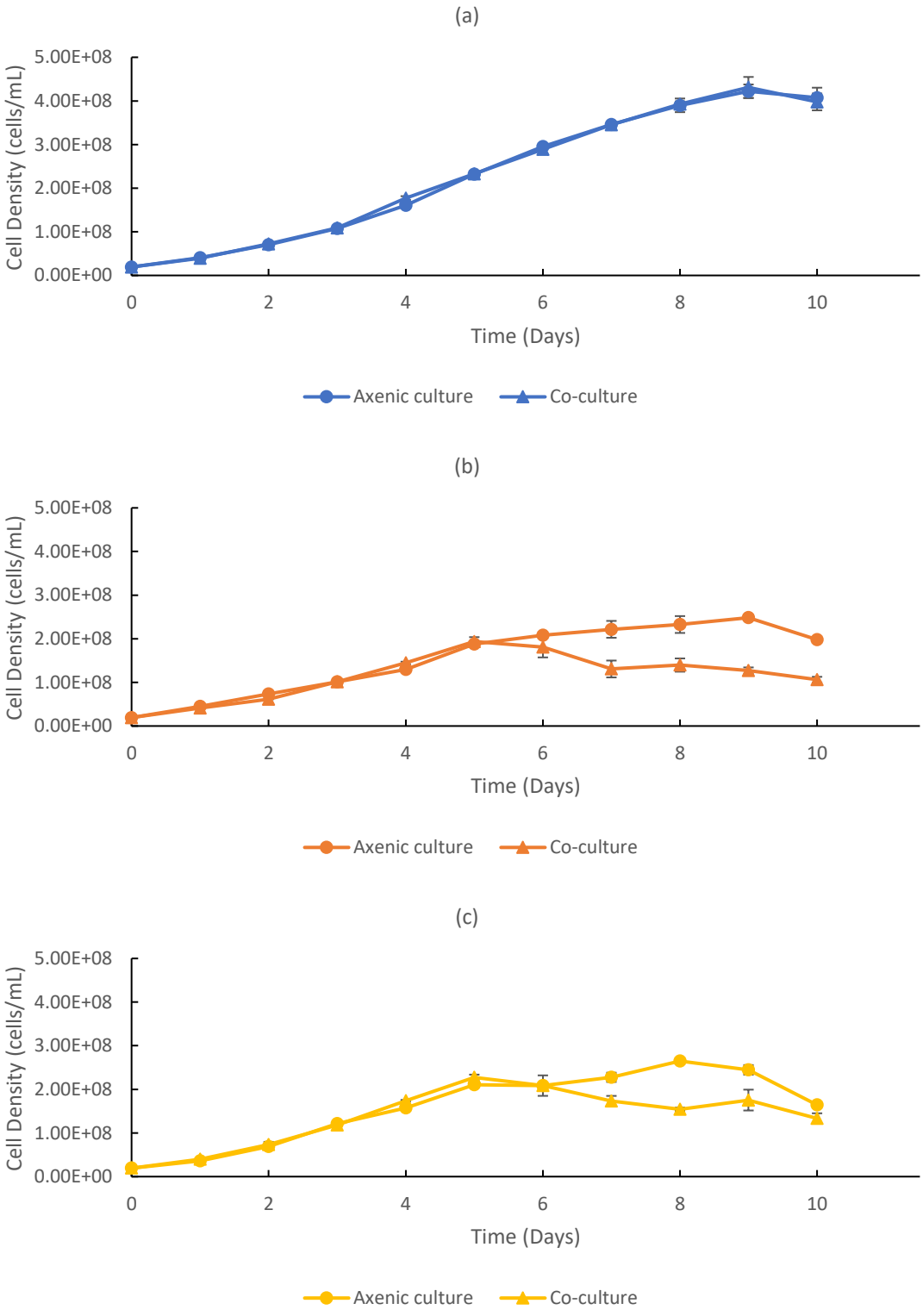


Figure 15. **Co-culture's effects on *S. elongatus* cscB.**

(a) Growth in control medium. (b) Growth in control medium with phosphate added at day 0. (c) Growth in control medium with phosphate added at day 3. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimtal groups are with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. *S. elongatus* cscB growths are monitored by measuring optical density at 750 nm.

Based on Fig. 15(b) and 15(c), the co-culture systems show much faster cell death rate and it happens earlier, comparing to axenic cultures. It is the opposite of the assumption, that the growth of *E. coli* W in co-culture should reduce the toxic effects of phosphate on *S. elongatus* cscB, as *E. coli* W may consume most phosphate as it grows. On the contrary, *S. elongatus* cscB cell density already starts to decrease at day 5 and is only at about 50% cell density of axenic culture when comparing cell densities at day 10 and cell density of axenic culture does not decrease until day 9. The result shows that the high phosphate concentration required by *E. coli* W is possibly not for cell consumption, but other purposes, or the excretion of *E. coli* W may be toxic to *S. elongatus* cscB, or both could be true.

The neglectable difference of growth in axenic culture and culture in control medium, Fig 15(a), is most likely the result of the low *E. coli* W growth, so the co-culture behaves more like axenic culture.

3.7.2 *E. coli* W growth in co-culture

Maximum cell density	Control	Day 0 phosphate addition	Day 3 phosphate addition	HEPES negative culture	Δpgi	ΔpflB
Co-culture	1.02×10^8	1.28×10^9	1.17×10^9	1.03×10^9	9.83×10^8	6.83×10^8
Growth in Fold	**7**	**86**	**78**	**69**	**59**	**39**
Axenic culture	/	1.87×10^9	1.87×10^9	1.62×10^9	2.10×10^9	5.00×10^9
Growth in Fold	/	**124**	**124**	**108**	**126**	**288**
Percentage (Co-culture/Axenic)	/	**68.8**	**62.5**	**63.9**	**46.8**	**13.7**

***Table 2*. *E. coli* W growth in axenic culture vs co-culture**.
Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups are with 32.16 mM phosphate added at day 0 or 3. HEPES Negative contains no HEPES with day 3 phosphate addition and others contains 3 g/L HEPES. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. Axenic culture contains 3 g/L sucrose as carbon sucrose. *E. coli* W is inoculated into co-culture system at day 3. *E. coli* W growths are monitored by plating diluted samples on LB agar plate.

Although growing significantly in co-culture systems, the *E. coli* W can only grow to 60% to 70% of the cell density in axenic cultures. This suggest that co-culture system puts extra stress on *E. coli* W. Also, it is worth noting that both knockouts tested show even worse than wild type, despite perform similarly in supernatant tests. More about knockouts test's result can be found in section 3.9.

3.7.3 Co-culture's impacts on sucrose production

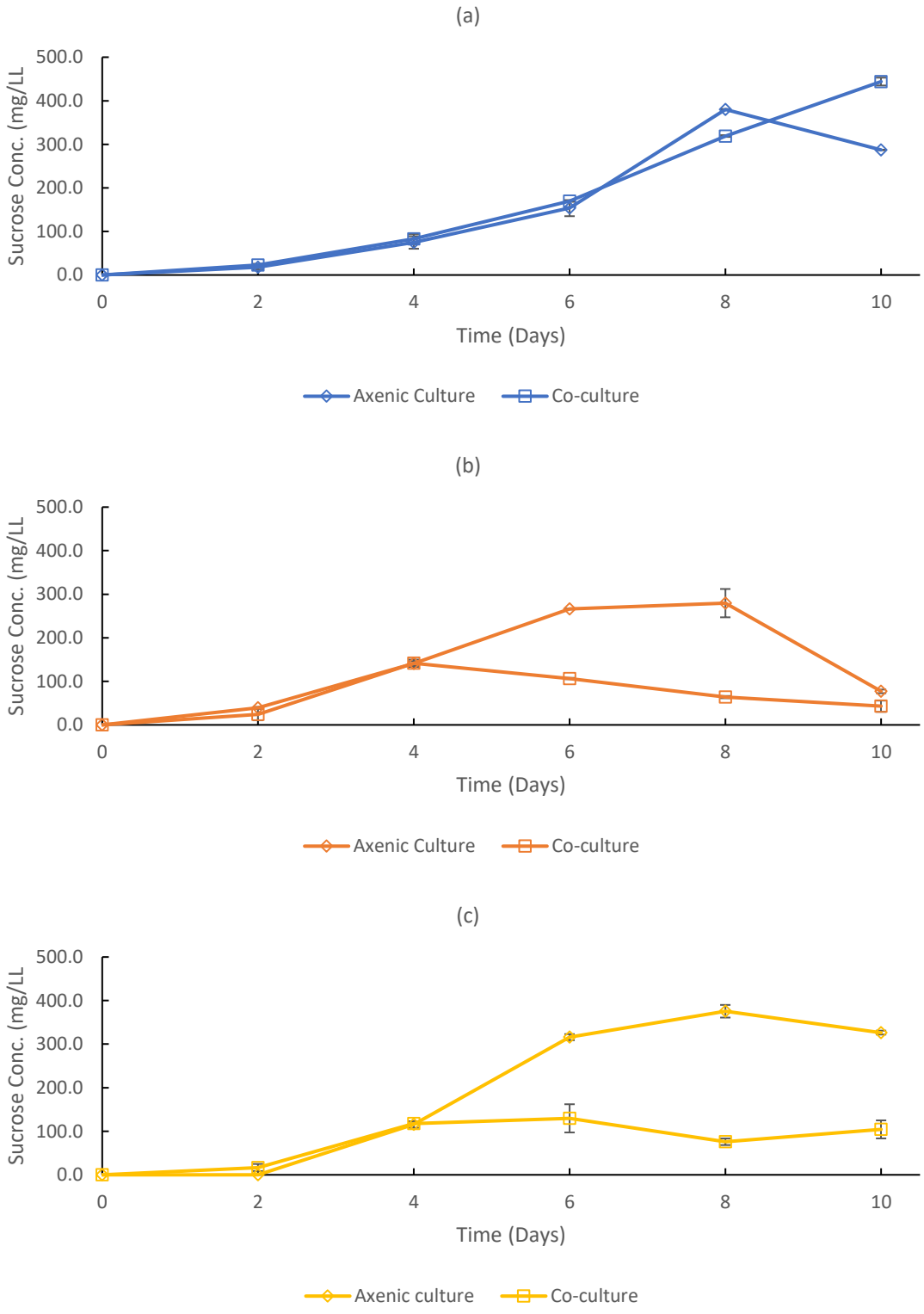


Figure 16. **Co-culture's effects on sucrose production.**

(a) Cultures in control medium. (b) Cultures in control medium with phosphate added at day 0. (c) Cultures in control medium with phosphate added at day 3. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimental groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. Sucrose concentration are measured by YSI 2700 SELECT.

The sucrose production and consumption in different mediums are as expected, in Fig 16(b) and 16(c), that as *E. coli* W starts to grow, the sucrose concentration stops increasing. Also, in all testing condition, *S. elongatus* cscB does not produce significant amount of sucrose until day 3 or 4, which is the reason to delay *E. coli* W inoculation until day 3 to avoid starvation of *E. coli* W.

The sucrose production rates are similar in axenic culture and co-culture of control medium, shown Fig 16(a), should be contributed to the low growth of *E. coli* W in the systems.

3.7.4 Co-culture's impacts on pH

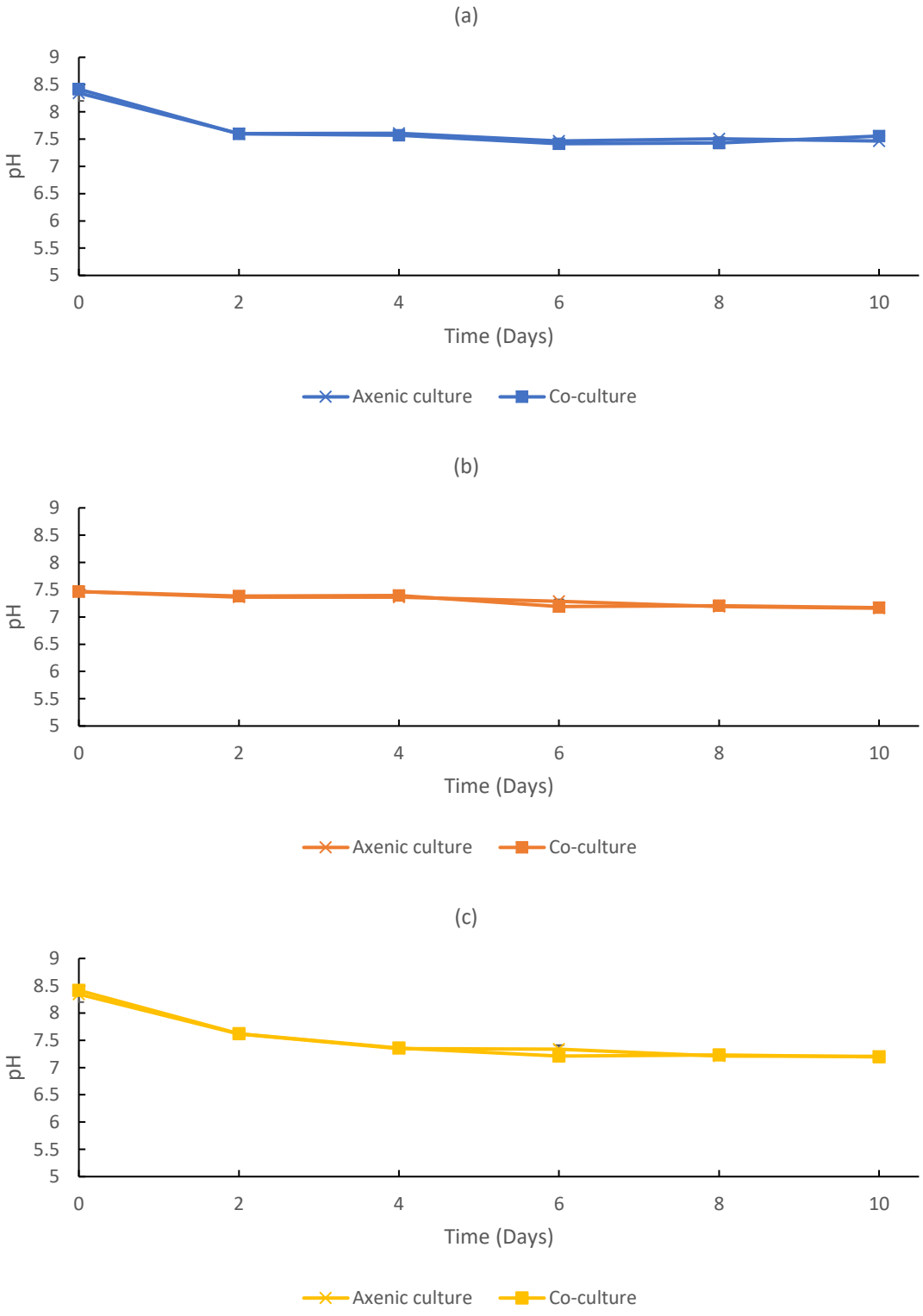


***Figure 17*. Co-culture's effects on pH**

(a) Cultures in control medium. (b) Cultures in control medium with phosphate added at day 0. (c) Cultures in control medium with phosphate added at day 3. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimtal groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. pH is measured by Orion Star A211 pH meter from Thermo Scientific with Orion 9110DJWP double junction pH electrode from Thermo scientific.

As Fig. 17 shown, the difference in pH of axenic culture and co-culture is neglectable in all scenarios. It shows that there may be significant changes in most likely *E. coli* W metabolic pathways or in *S. elongatus* cscB to overcome *E. coli* W's effect on pH, evidenced by that *E. coli* W axenic culture typically reach a very low pH 5.3, shown in 3.1, and that pH remains basic in co-cultures, although the neglectable difference in control medium tests should be contribute to the low *E. coli* W growth, Fig 17(a).

3.7.5 Co-culture's impacts on ammonium consumption

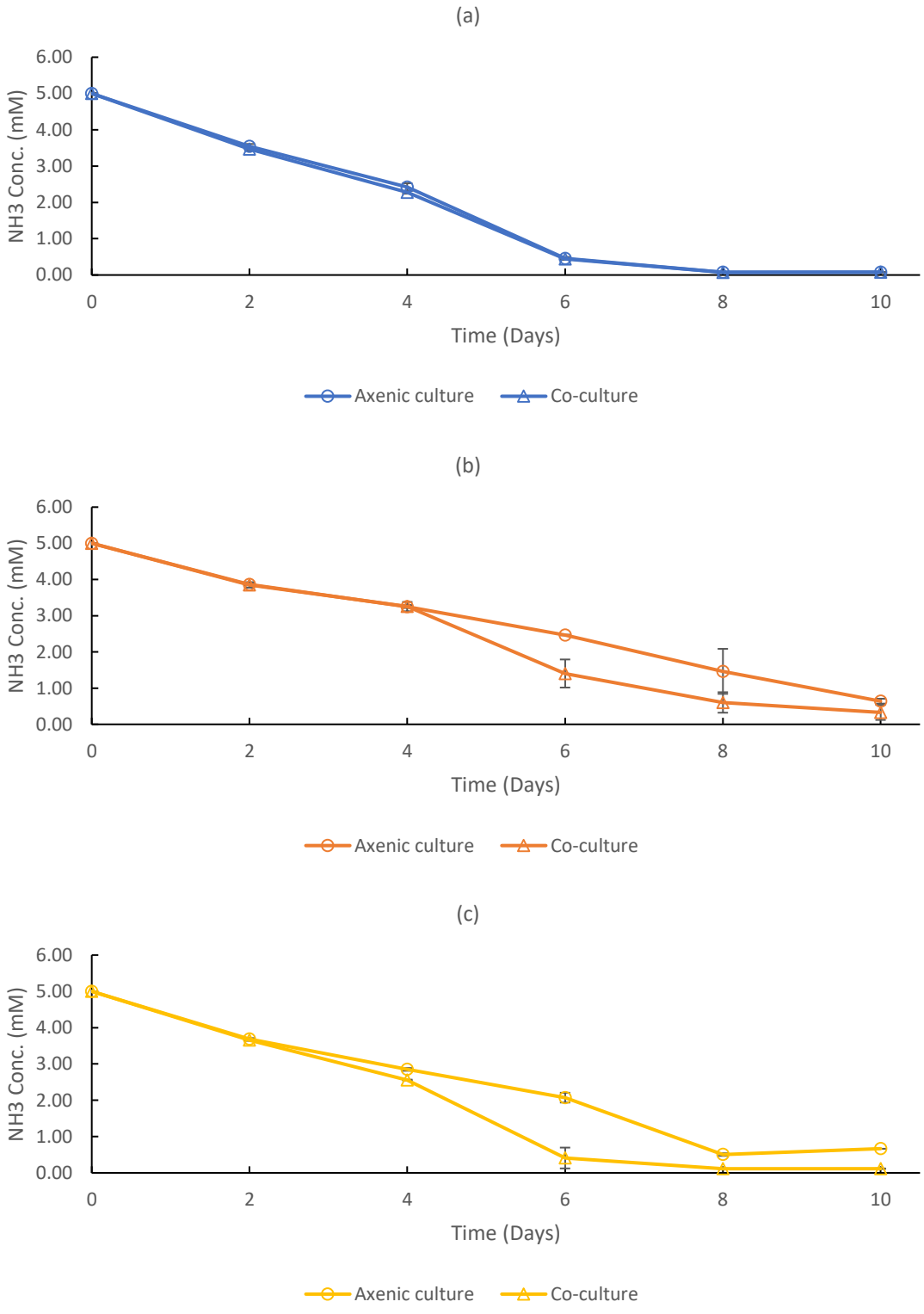


***Figure 18*. Co-culture's effects on ammonium consumption.**

(a) Cultures in control medium. (b) Cultures in control medium with phosphate added at day 0. (c) Cultures in control medium with phosphate added at day 3. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimtal groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 μmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. The ammonium concentration is measured by using a reagent assay containing 5 mL of ethanol, 270 mg phthalic dicarboxaldehyde, and 50 μL of β-mercaptoethanol in 100 mL of 0.2 M phosphate buffer with a pH =7.3.

As shown in Fig. 17(b) and 17(c), the ammonium utilization starts to increase in cocultures at day 3, comparing to axenic culture, due to the inoculation of *E. coli* W and ammonium utilization rate after day 3 is much faster in day 3 phosphate addition co-culture trails then in day 0 phosphate addition, suggesting that the timing of phosphate addition has impacts on ammonium utilization, which is also shown in section 3.6.5.

3.8 Phosphate consumption

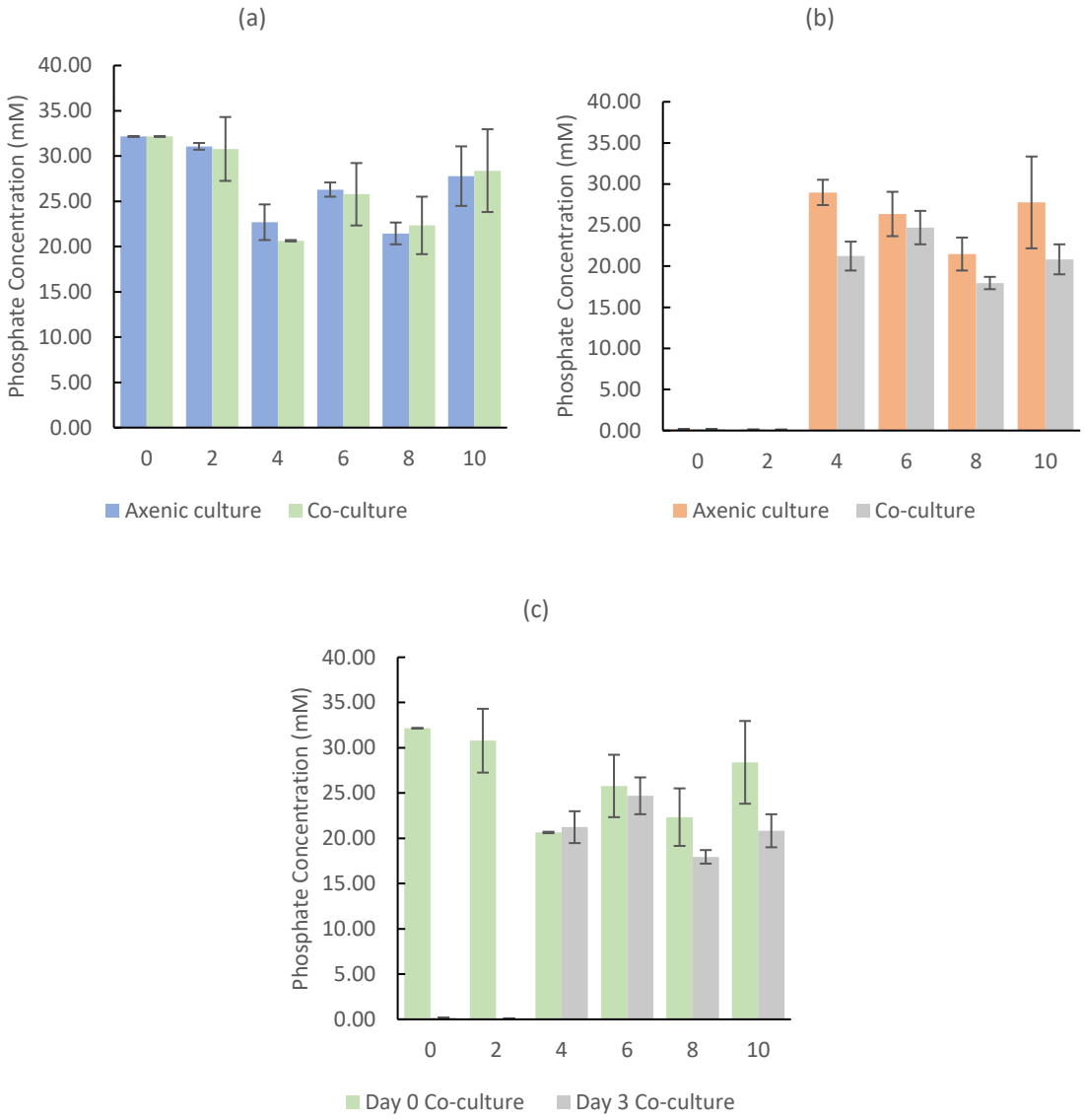


***Figure 19*. Phosphate consumption in phosphate addition medium.**
(a) Phosphate added at day 0 (b) Phosphate added at day 3. (c) Comparison in between day 0 phosphate addition co-culture and day 3 phosphate addition co-culture. Control medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG and experimtal groups with 32.16 mM phosphate added at day 0 or 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. Phosphate concentration are measured with 10 mL diluted sample, 0. 2 mL 10% (w/v) ascorbic acid, and 0.4 mL of reagent contenting 130 g/L ammonium molybdate, 3.5 g/L antimony potassium, and 30 % (v/v) sulfuric acid, react for 15 min the measure absorbance at 700 nm.

As suggested in section 3.7.1, the high amount of phosphate may not be consumed by *E. coli* W, the phosphate concentration profile over the course of the co-culture trial is investigated to confirm it.

Fig. 19(c) provides the conformation that *E. coli* W, although consumed some phosphate, most of the phosphate is still presents in medium. In addition, the *S. elongatus* cscB also consumes some phosphate starting from day 3 or day 4, which reduces the phosphate concentration to 30 mM from 32.16 mM in axenic cultures, shown in Fig. 19(a) and 19(b). It is noticed that as *S. elongatus* cscB starts dying at day 8, the phosphate concentration rises again on the same day. It is possible that *S. elongatus* cscB uptakes the phosphate into the cell but cannot incorporate them into cellular activities.

3.9 Co-culture of selected *E. coli* W knockouts

3.6.1 *S. elongatus* cscB growth with different knockouts

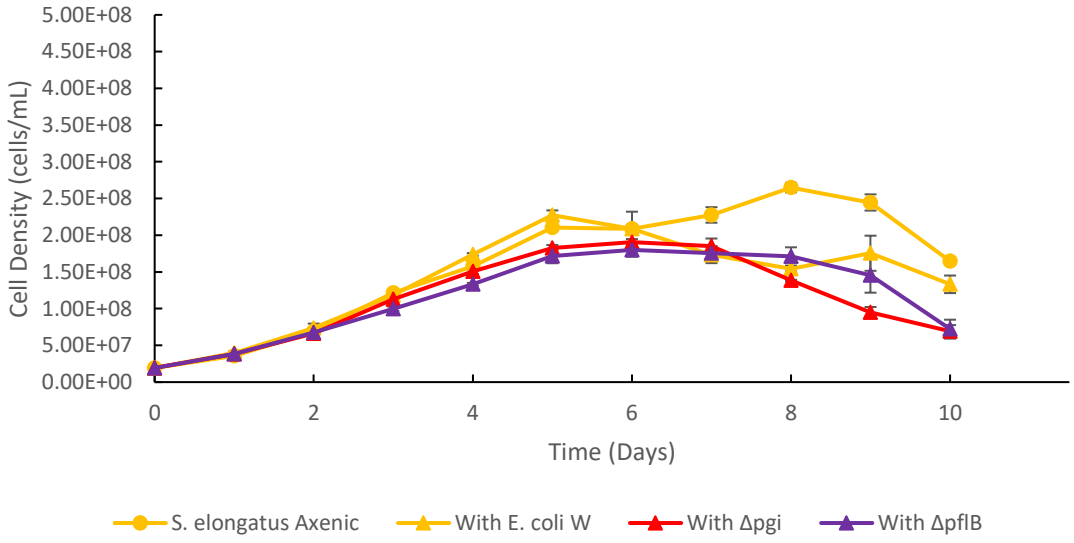


***Figure 20*. *S. elongatus* cscB growth in co-cultures with *E. coli* W and its knockouts.**
Medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG added, and 32.16 mM phosphate added at day 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, *with 1% CO_2/Air bubbling continuously*. *E. coli* W is inoculated into co-culture system at day 3. *S. elongatus* cscB growths are monitored by measuring optical density at 750 nm.

Based upon the result of supernatant growth tests, shown in section 3.3, ΔpflB and Δpgi are chosen to be tested in co-culture system.

S. elongatus cscB behaves similarly when co-cultures with wildtype and two knockouts and shows signs of additional growth stress when compared with axenic culture as also demonstrated in section 3.7.1. Despite showing promising result in supernatant tests, ΔpflB and Δpgi do not any show positive effects on *S. elongatus* cscB growth but also do not show any additional negative effects either, comparing with the wildtype.

3.6.2 *E. coli* W and knockouts growth in co-culture

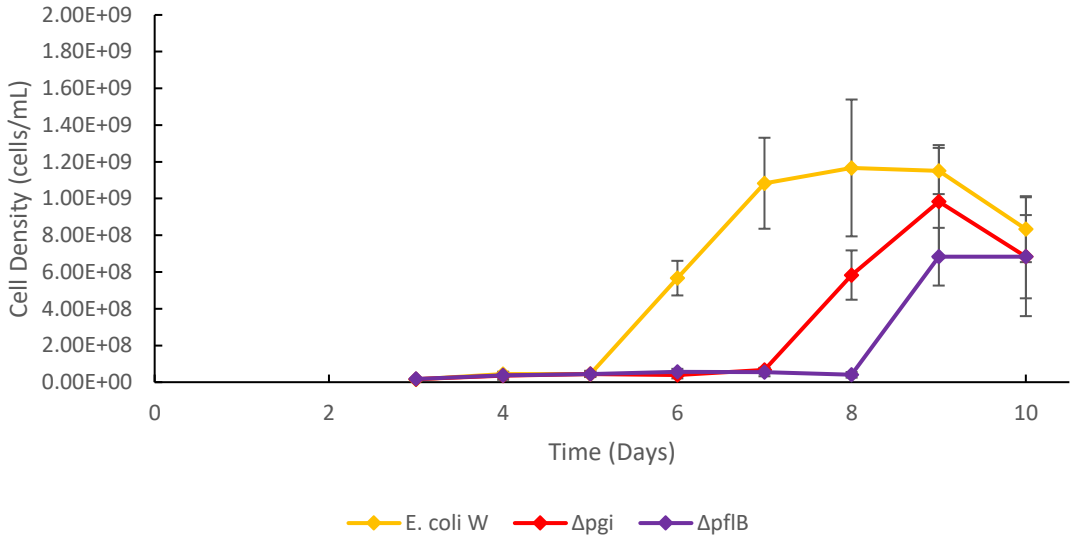


***Figure 21*. *E. coli* W and knockouts growth in co-cultures.**
Medium is BG-11 medium with 100 mM NaCl, 5 mM NH_4Cl, and 1 mM IPTG added, and 32.16 mM phosphate added at day 3. All experiments are performed at 28 °C temperature, 16:8 hours light/dark cycle and 100 µmol/m^2/s light intensity produced by cool-white fluorescent lamps, with 1% CO_2/Air bubbling continuously. *E. coli* W is inoculated into co-culture system at day 3. *E. coli* W growths are monitored by plating diluted samples on LB agar plate.

As mentioned in section 3.7.2, the chosen knockouts, ΔpflB and Δpgi, shows lower maximum cell density than the wild type. In addition to that, Fig. 21 also shows that ΔpflB and Δpgi has a significantly longer lag phase than the wildtype; Δpgi does not start show significant growth until day 7 and ΔpflB until day 8. Although both show longer lag phase in axenic culture tests too, it is only about 8 to 12 hours instead of several days.

DISCUSSION

E. coli W's metabolic wastes showed clear inhibitory effects on *S. elongatus* cscB growth. Comparing the growth in Fig. 3(a) and Fig. 4(b), *S. elongatus* shows no growth in most supernatants collected with various *E. coli* W knockouts and very limited growth in Δpgi and ΔpflB; the cell density of the highest growth in supernatant reaches only roughly 1/3 of the growth in the comparable medium shown in Fig. 3(a). It is a reasonable argument that the poor growth in *E. coli* W supernatants may be partially contributed to the left-over sucrose in the supernatants, but the lower maximum cell density and earlier entering of stationary and cell death phase in co-culture systems comparing with axenic culture systems is a more clear indication of the inhibitory effects.

Also worth noting, the relative high growth in ΔpflB and Δpgi supernatants, shown in Fig. 3(a), could be due to the metabolite exported by these two knockouts, since both genes code enzymes involved in cell energy production thus, the metabolite products from them could contain higher amount of energy and be utilized by *S. elongatus* cscB for improving growth rate. It suggests some heterotrophy capabilities of *S. elongatus* cscB and chance to use *E. coli* W metabolites to improve *S. elongatus* cscB growth in co-culture.

On the opposite side, it is possible that the co-culture systems have a high sucrose production rate comparing with *S. elongatus* cscB as suggested in section 3.5.3, which could be a result of the consumption of sucrose in medium by *E. coli* W or metabolic changes in *S. elongatus* cscB. It has been reported that the engineered *S. elongatus* cscB has higher photosynthesis efficiency than wild type *S. elongatus*.(Abramson et al., 2016) The interactions

with of *E. coli* W may further improve the efficiency or just alter metabolic reactions in *S. elongatus* cscB towards sucrose production and exportation.

As demonstrated in the result section, *E. coli* W's growth is suspected to be limited by pH in axenic culture and there are studies to prove that *E. coli* W growth rate decreases with rising hydrogen ion concentration, in another words, lower pH. (Presser, Ratkowsky, & Ross, 1997) However, this factor is not accountable for the limited growth of *E. coli* W in co-culture. Although pH is considered because the sucrose production of *S. elongatus* cscB also decreases with the pH in addition of *E. coli* W growth since sucrose permease encoded in cscB gene co-transport hydrogen ions. However, in all scenarios including HEPES negative test, the pH of co-culture systems remains above 6.0 at all time and stabilized at around 7.0 or above at end of the trails, which is higher than the average final pH 5.3 of *E. coli* Q axenic cultures.

On further inspections of the data, the limiting factor in co-culture could be ammonium concentration in medium and possible inhibitory effect form *S. elongatus* cscB. Ammonium has not been considered as a limiting factor as it is not a required nutrient for *S. elongatus* cscB, for example the medium used for generating stock solution consisting of no ammonium. However, as shown in Fig. 13(a) and 18, *S. elongatus* cscB consume considerable amount of ammonium in axenic cultures as well, thus competes with *E. coli* W. Although there are nitrates available in medium at 17.65 mM, based on the medium used, the utilization of nitrate in *E. coli* W is at the expense of hydrogen ion, which could be a reason why co-culture pH is significantly higher than *E. coli* W axenic culture, and this process produces nitric oxide which is toxic to some strains. (ATKINSON & MCNALL, 1956) On other note, the axenic culture of *S. elongatus* cscB and co-cultures have similar trend for ammonium consumption, which could suggest a reduction in ammonium consumption of *S. elongatus* cscB in co-culture, however it is not a certainty due to

the lack of *S. elongatus* cscB growth and *E. coli* W growth. Also, the pH difference in axenic *E. coli* W and co-culture is too great be only caused by nitrate utilization and could be a result of metabolite exchanges between two microorganisms.

The phosphate addition is major alteration in medium from *S. elongatus* cscB axenic culture to co-culture and the primary obstacle in stabilizing the co-culture system. Based upon data collected so far, such a high phosphate concentration is required for *E. coli* W growth and has inhibitory effects on *S. elongatus* cscB, which has been expected. However, the low phosphate consumption in medium is not expected. It has been studied that *E. coli* strains do not actually consume phosphate but rather use high exocellular phosphate concentration to maintain a favorable intercellular phosphate concentration for catalyzing cellular reactions.(Willsky, Bennett, & Malamy, 1973) Also, the low growth of *E. coli* W in low phosphate concentration medium is likely due to low cellular reactions rates caused by low phosphate concentration.

On the positive side, phosphate addition increased the sucrose production rate of *S. elongatus* cscB, and it could be because that cscB gene is a *E. coli* gene. When the medium is improved for *E. coli* W growth, it also optimized the condition for sucrose permease coded by cscB gene, thus increase its efficiency. However, this could also contribute to the slow growth of *S. elongatus* cscB. The sucrose production is triggered to combat osmotic pressure and increasing exportation may cause some cells to lose the ability to control their cellular volume thus leading to slow growth and cell death. Without further studies in enzymes' activities in *S. elongatus* cscB under phosphate positive and negative conditions, it is hard to confirm such theory.

For concerns about the phosphate addition altering the osmotic pressure of the medium, *S. elongatus* cscB has been proven to grow in medium with 200 mM NaClo instead of 100 mM.

(Ducat et al., 2012) Tests are also performed although not shown in this thesis with 150 mM NaCl *S. elongatus* cscB stock inoculated in 100 mM NaCl medium with phosphate addition vs 150 mM NaCl without phosphate addition and 100 mM *S. elongatus* cscB stock inoculated in 50 mM NaCl medium with phosphate addition vs 100 mM NaCl without phosphate addition. There is difference in growth rate, but phosphate addition trails always grow slower than corresponding control trails. So, the phosphate's toxicity on *S. elongatus* cscB is clear.

CONCLUSION

A co-culture system consisting of *S. elongatus* cscB and *E. coli* W is feasible but may not be favorable, due to the dramatic difference in nutrient requirements. Further work is needed to stabilize the co-culture system and turn it into a usable production system.

FUTURE WORK

This thesis only provides a feasibility study and an initial attempt for building a workable co-culture system of *S. elongatus* cscB and *E. coli* W. It enlightens the future of such a system and revels what future study should focus on.

6.1 Metabolic analysis of axenic culture and co-culture systems

Due to the changes in medium and evidenced by data presented above, there may be significant changes in the metabolic pathways within both microbes. In addition, there are more interactions between *S. elongatus* cscB and *E. coli* W than only sucrose, O_2 and CO_2 exchanges. A complete metabolic analysis is crucial for understanding those changes and interaction between them and should focus on metabolic products excreted from both microorganisms; the toxicities of those metabolic products on both microorganisms should be studied too.

6.2 Construction of an accurate genome scale model

Genome scale model can simulate the metabolic responses of different microorganisms under different constrains and potentially their interactions (Smallbone, Simeonidis, Swainston, & Mendes, 2010), and has been used for co-culture systems before with great effects (Zuniga et al., 2016), so an accurate genome scale model of the co-culture system could further the understanding in the metabolic changes reveled in metabolic analysis. The genome scale model can also be used to guide feeding options and further medium optimizations too. For example, the feeding of phosphate could be controlled by model based on *E. coli* W cell growth thus maintain a relatively low phosphate concentration in medium that both sustains *E. coli* W growth and reduce the inhibitory effects on *S. elongatus* cscB.

6.3 Use genetic tool to produce suitable mutants for co-culture

As mentioned in some researches, specific genes related to phosphate transportation in *E. coli* W are activated at low phosphate concentration. (Surin, Rosenberg, & Cox, 1985) By over expressing such genes, *E. coli* W could be potentially enabled to grow in low phosphate concentration. Genetically engineering more suitable phosphate transporters into *E. coli* W or modifying *S. elongatus* cscB to be suitable for high phosphate concentration are also options.

In addition of co-culture tests listed above, other gene knockouts of *E. coli* W and *S. elongatus* cscB should be tested to confirm the result of metabolic analysis, examine the accuracy of the genome scale model and may also be used to reduce the toxic effects of *E. coli* W and *S. elongatus* cscB metabolic wastes on each other.

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Education

Johns Hopkins University, Baltimore, MD
Master of Science, Chemical and Biomolecular Engineering — Excepted May 2019
GPA 3.58/4.00
Michigan State University, East Lansing, MI
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GPA 3.46/4.00
Shandong Normal University, Jinan, Shandong, China — Sep. 2010- Jun. 2011
Major in Business Administration, no degree awarded
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Certification

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Research

Biosystems Engineering Senior Design Project, Michigan State University, East Lansing, MI (Oct. 2016 – May 2017)

- A two-semester team project for Dow AgroSciences to develop a pilot scale composter as a testing equipment for tracking chemical degradation
- Background research on composting principles, feedstock material, and composter designs
- Prototype design and build to meet specific feedstock requirements, data collection requirements and sampling for tracking chemical degradation
- Data collection through programmed Campbell CR-1000 data logger and required sensors to for continuously online data collection in month long trails
- Chemical degradation is tracked by running collected samples through MSR

Master Thesis Research Project – Cell Coculture Project, Johns Hopkins University, Baltimore, MD (Dec. 2017 – Present)

- JGI and EMSL funded project to build a synthetic autotrophic-heterotrophic system
- Background research on microorganisms involved, specifically a engineered sucrose secreting *S.elongatus, E. coli* W, *E.coli* K-12 and their knockouts
- Genome-scale model for the coculture system is built and used to guide the research
- Proved the concept and optimized the medium via batch and semi batch reactor experiments for medium optimization, which is the key for this coculture system, and data collection

- Various methods and equipment for data collection: flow cytometry, chemical assays, YSI, HPLC and so on

Graduate Research Project – In situ Online SERS Probe, Johns Hopkins University, Baltimore, MD (Dec. 2017 – Present)

- AMBIC funded project to optimize current Raman spectroscopy probe for in-situ and online monitoring of cell culture metabolites via nanomaterial surface enhancement
- Background research on principles of Raman spectroscopy and surface-enhanced Raman spectroscopy, experiment methods and data processing methods
- Both Raman spectroscopy and SERS are tested; both commercial and specifically design SERS substrate are used in testing
- MATLAB coding and data processing software like Origin are used to process the data collected
- Proved the concept of the project and the possibility of quantification with SERS through comparing detect limitation, signal to noise ratio and so on

Graduate Research Project – Integrating Chemical and Biological Processes for Deriving Value-Added Products, Johns Hopkins University, Baltimore, MD (Sep. 2018 – Present)

- A BEEPS funded project to connect chemical catalysis reactor with bioreactor to produce value-added products from carbon dioxide
- Background research in electrochemical reduction of carbon dioxide and suitable microbe strains for utilizing carbon intermediate from chemical catalysis
- In concept proving stage

Publication

Li, T., Jiang, L., **Hu, Y.**, Paul, J. Zuniga, C., Zengler, K., Betenbaugh, M. J., Mutualistic co-culture of fungi and extracellular polysaccharide-secreting cyanobacterium *Nostoc* PCC 7413, submitted.

Wang, Q., Chen, Y., Park, J., Liu, X., **Hu, Y.**, Wang, T., McFarland, K., Betenbaugh, M., Design and biomanufacturing of bispecific antibodies, *Antibodies*, submitted.

Experience

Undergraduate Learning Assistant for Basic Physics, Michigan State University, East Lansing MI (Jan. 2017 – May. 2017)

- Special session for students from biology related majors
- Active learning style class
- Help develop class materials and exams
- Lead group discusses
- Hold office hours

Volunteer Work

Chinese Students & Scholars Association of Mich. State University, East Lansing, MI (Sep. 2012 – May 2)

- Photographed events (5 events per year)
- Presented for university freshman in China

Lewton Elementary School, Lansing, MI (Feb. 2012 – May 2012)

- Tutor math and English
- Taught basic math and reading

Skills

- Basic knowledge in AutoCAD and MATLAB
- Chemical and biochemical laboratory techniques
- Trained in flow cytometer, HPLC and YSI
- Basic knowledge in Microsoft Word, PowerPoint, and Excel